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## CONTENTS



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# METASTATIC OR EMBOLIC ENDOMETRIOSIS, DUE TO THE MENSTRUAL DISSEMINATION OF ENDOMETRIAL TISSUE INTO THE VENOUS CIRCULATION*

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Over twelve years ago the writer began a series of experiments to determine the shape of the uterine cavity in normal and pathologic conditions. The technic was as follows. The uterus removed at operation or necropsy was placed in a basin of warm water and then filled with melted gelatin (about 15 per cent) containing in suspension bismuth subcarbonate or barium sulphate. This was introduced through the cervical canal by means of a syringe. After filling the uterine cavity the syringe was withdrawn, the cervix clamped in order to prevent the escape of the injection mass and the specimen placed in cold water until the gelatin had solidified. Stereoscopic roentgenograms of the uterus enabled one to obtain a clear picture of the form of the uterine cavity under various conditions and also of the lumina of the tubes if the latter were patent. In February, 1916, I removed a myomatous uterus from a patient who was menstruating at the time of the operation. On filling the uterine cavity with the injection mass I was surprised to find that it escaped from the severed uterine and ovarian veins. This was the first time that I had noticed this phenomenon. The following experiments were made. Uteri were curetted after their removal and the uterine cavity was filled with the mass. In many instances the injection mass escaped into the venous sinuses of the uterine wall and through the uterine and ovarian veins. These observations together with a

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description of the venous circulation of the uterus, based on the study of uteri in which the veins had been injected, were published in the year 1918 in an article [1] dealing with the escape of foreign material into the venous circulation of the uterus. It then seemed to me that bits of the uterine mucosa, occasionally, might escape into the venous circulation during menstruation. I have frequently asked pathologists if they have ever found endometrial tissue in the lungs of women but have not ascertained that it has ever been observed.

As a result of these studies it is natural that I should consider the menstrual changes in the uterine mucosa a means of the dissemination of bits of endometrial tissue into the uterine circulation and as a possible source of certain instances of misplaced endometrial tissue in that organ and outside of it. To me it was a most plausible theory but lacked proof. I believed, however, that it must occur and could be demonstrated. It was just a question of a better knowledge of the venous and lymphatic circulations of the uterus and the histologic study of many sections of uteri, especially of those removed during menstruation.

### The Intrinsic Blood Supply of the Uterus

The blood supply of the uterus was studied by injecting the vessels with melted gelatin containing bismuth subcarbonate and hardening the specimen in formalin. Stereoscopic roentgenograms were made of the entire uterus and also of cross slices of the same. The fine branches were studied in microscopic sections.

The general plan of the distribution of the intrinsic arteries of the uterus is as follows. Branches arise in pairs from each uterine artery and opposite each other, one branch penetrating the anterior and the other the posterior uterine wall. These two pairs of branches, which may be called arcuate arteries on account of their course in the myometrium, divide the uterine wall into a narrow outer or peripheral zone nourished by the peripheral branches of these arteries and a wide, inner or radial zone supplied by their radial branches (so named on account of their course). The latter terminate in the endometrium. The greater portion of the arterial supply of the uterus is directed toward its mucosa (Fig. 1). Each pair of arcuate arteries supplies a segment of the uterus corresponding to a segment of the Müllerian duct of that side.

The venous blood is conveyed from the uterine tissues mainly by the arcuate veins (corresponding in their general course with that of the arcuate arteries) which empty into the uterine plexus of veins, situated between the layers of the broad ligament. The arcuate veins receive blood from both the peripheral and the radial zones of the uterus (Fig. 2). The venous capillaries of the mucosa, some of which are dilated forming sinuses (Fig. 3), empty into the venous sinuses of the radial zone of the myometrium and these in turn into the arcuate veins. Some of the former are relatively large and radiate from the endometrium (Fig. 10). I have named these sinuses radial or, better, receiving sinuses, as they receive the greater portion of the blood from the endometrium and are continuous with the dilated capillaries (sinuses) of the latter. It is obvious that foreign material gaining access to the mucosal sinuses might escape through the receiving sinuses into the deeper vessels of the uterine wall. As there are no valves in the sinuses and veins of the uterine wall, the various physiologic changes in the venous pressure in these vessels might readily force foreign material in them into any of the sinuses and veins of the uterine wall, including those of the peripheral zone (Fig. 11), as they are to one side of the main blood stream which passes through the radial zone into the arcuate veins and thence to the uterine veins between the layers of the broad ligament. Only the principal veins of the uterus have well defined walls; the greater number of them, including the receiving sinuses, are usually only endothelial-lined spaces between the muscle bundles. In the non-injected specimen the veins and venous sinuses are often empty or contain very little blood and are therefore readily taken for lymphatics.

At menstruation some of the capillaries of the mucosa rupture and blood escapes into the tissues of the latter (Figs. 6, 7 and 8). Often bits of the mucosa may be found lying free in this extravasated blood. It is natural to assume that at times some of these bits might escape into the lumen of a ruptured mucosal capillary or sinus and be carried through a receiving sinus into the deeper vessels of the uterine wall. This does occur as will be shown.

The histologic study of the ectopic endometrial tissue in a direct or primary endometriosis (so-called adenomyoma of mucosal origin) shows that this tissue contains venous capillaries (Fig. 12) similar to those of the mucosa lining the uterine cavity (possibly not so

large) and furthermore that this tissue, in its invasion of the myometrium, often extends in the spaces occupied by the vessels and sinuses of the uterine wall but is separated from the lumen of the latter by the endothelial lining of the vessel (Figs. 14, 15, 16, 17 and 18), as has been emphasized by Robert Meyer in his description of the relation of this ectopic endometrial tissue to the lymphatics of the uterine wall. (I believe, however, that the majority of these vessels are veins and not lymphatics.) It might be assumed that in the menstrual reaction of this misplaced endometrial tissue, bits of it might escape into its venous capillaries and also possibly into the lumen of a sinus of the uterine wall along which the endometrial tissue often extends in an extra- or retro-endothelial course. I also believe that the same applies to misplaced endometrial tissue of other origin than from a direct invasion of the uterine wall by its mucosa (Fig. 13). In one instance of an endometriosis of the cul-de-sac presenting in the posterior vaginal vault (a so-called adenomyoma of the recto-vaginal septum) the actual escape of the menstrual contents of two ectopic endometrial cavities into adjacent veins was found (Figs. 60 and 61) and, furthermore, bits of endometrium were present lying free in the lumina of and implanted on the lining of veins about these cavities (Figs. 58, 59 and 66). As a result of this finding I believe that a similar condition occasionally must arise in a direct endometriosis of the uterine wall. I have not definitely demonstrated it after a careful study of many sections from many blocks of tissue from uteri with a direct endometriosis, which were removed during the menstrual period, but believe that either it has been seen by others or will be found.

As bits of the uterine mucosa at times escape into the venous circulation of the uterus during menstruation, certain questions naturally arise. What is its pathologic and clinical significance? Could it possibly give rise to ectopic endometrial tissue not only in the uterine wall but also outside of that organ?

## The Lymphatic Circulation of the Uterus

Is it possible for bits of the uterine mucosa to be disseminated through the lymph vessels during menstruation, or may it actually invade these vessels and pieces of it escape through these channels? I have attempted to inject the lymphatics of the uterine wall and its mucosa but failed. Vessels which I have previously considered

lymph vessels have corresponded to the venous capillaries and sinuses of uteri in which the veins have been injected. The subperitoneal lymph vessels about the uterine cornua and between the layers of the broad ligament may often be easily recognized without injecting them; but when one attempts to inject the deeper lymphatics of the uterine wall the venous capillaries and sinuses often become filled with the injection medium and may readily be taken for lymph vessels because they have a structure similar to the latter.

In an article [2] published in 1922, I suggested that endometrial tissue might metastasize through lymph vessels, because I had found an endometrial polyp projecting into the lumen of a lymph vessel situated between the layers of the broad ligament. This polyp had arisen from the invasion of the vessel by endometrial tissue outside of the vessel pushing the endothelial lining of the lymphatic ahead of it. I also added that metastases might arise from the direct invasion of the uterine wall by the mucosa lining its cavity and from a similar invasion of the tubal wall by its mucosa.

In 1924 Halban published a preliminary communication [3] on the metastatic origin of misplaced endometrial tissue through the lymphatics. He believed that in the invasion of the myometrium by its mucosa some of the epithelium escapes into the lymph spaces between the muscle bundles of the uterine wall and is carried through these to the superficial lymphatics beneath the serosa and from there spreads by the lymph channels to other pelvic structures including the inguinal lymph nodes.

The following year I [4] discussed the possibility of metastasis of endometrial tissue through the lymph channels and suggested that the menstrual reaction of endometrial tissue encroaching upon or protruding into a lymphatic might disseminate bits of this tissue into its lumen and lead to metastasis. Robert Meyer, in a letter to me, justly criticised my suggestions and theories because I had not actually proved them. He referred to his own publications [5] and those of Kitai [6] where they had attempted to find evidence that endometrial tissue actually broke through the endothelial lining of the lymph vessels but had been unable to prove that it had. He well describes [7] the relation between the invading endometrial tubules and the lymph vessels and the way the former often follow these vessels and distort them without actually gaining access to the lumen

of the vessel. I can confirm Robert Meyer's observations as to the extra-endothelial course of endometrial tissue accompanying the vessels of the uterine wall in a direct endometriosis, but believe that the latter are usually venous sinuses and not lymph vessels. I also believe that the menstrual reaction of the endometrial tissue alongside of a vessel might cause rupture of the endothelial lining of the vessel and permit bits of endometrial tissue to escape into its lumen, but have not been able definitely to prove it.

Due to my inability to recognize the lymphatics of the uterine mucosa and the deeper tissues of the myometrium I have been unable to determine the part they take in the dissemination of endometrial tissue.

For anatomic and physiologic reasons it seems to me that metastases of endometrial tissue from the uterine mucosa and also from ectopic endometrial foci are more apt to be of venous than of lymphatic distribution. Still, this is a minor point which in time will be settled.

## Endometrial Tissue in the Venous Sinuses of the Uterine Wall due to Artefacts

If bits of endometrial tissue sometimes escape into the venous circulation of the uterus during menstruation, they should occasionally be seen in the veins and venous sinuses of uteri removed during the menstrual period. The problem would seem to be a very easy one. If they were not found after a careful study of many sections from many menstruating uteri it probably does not occur and if bits of endometrial tissue are discovered lying free in the lumina of these vessels the problem is solved. I encountered certain technical difficulties. The veins and venous sinuses of the uterus removed by operation are often empty or contain very little blood. The surgeon usually clamps the uterine side of the ovarian and uterine vessels and ligates the distal portion cutting between the clamps and the ligatures. After the uterus has been removed and the clamps released from the vessels the greater portion of the blood within the uterus escapes from the severed vessels and might carry with it any foreign material present in that blood. If the uterus is incised before it is fixed more blood escapes from its tissues. I partially obviated this difficulty by doubly ligating the vessels and

cutting between the ligatures before removing the uterus. The entire specimen was hardened in formalin and blocks were not cut from it until it was fully fixed. The uterus was cut into cross slices and these slices were cut in halves or quarters depending upon their size and embedded in celloidin. The majority of the veins and venous sinuses of the uterine wall were still found to be empty or to contain very little blood, but the uterine plexus of veins on either side of the uterus was distended with blood. During fixation the uterus evidently contracts and forces the greater portion of the blood in its sinuses into the veins on either side of it.

Bits of uterine mucosa were found in the veins and sinuses of the wall of menstruating uteri. A careful study of these sections and the process of embedding showed that some of these findings were due to artefacts. The menstruating uterine mucosa is very friable; bits of it break off during the process of embedding and readily drop into the empty veins and sinuses of the uterine wall. These appear as endometrial emboli in the stained sections (see Figs. 19, 20 and 21). If, however, pieces of the uterine mucosa are found surrounded by blood in a vein or sinus or attached to the wall of the vessel by fibrin, it is evident that they reached this situation before the tissues were fixed. It is also possible that bits of the uterine mucosa may be carried into the venous sinuses of the uterine wall in cutting blocks from the unfixed uterus.

## Endometrial Tissue in the Veins and Venous Sinuses of the Uterine Wall Due to the Menstrual Dissemination of this Tissue into these Vessels

Fragments of endometrial tissue were found either in the blood of the veins and venous sinuses of the uterine wall or attached to the lining of these vessels by fibrin in three uteri removed during the menstrual period (Cases 1, 3 and 4). In two other uteri, removed during the menstrual period, clumps of epithelium-like cells which I was unable to identify, were found in the blood or attached to the walls of veins. A careful study of three other uteri removed during menstruation failed to reveal any embolic endometrial tissue in the vessels of the uterine wall but several artefacts were present in sections from one uterus (see Figs. 19*a*, 20 and 21). Sections were carefully examined for endometrial tissue in the veins and venous

sinuses of many uteri removed at other times than during menstruation, and embolus-like lesions of endometrial tissue were found in only one uterus (see Case 2).

The histologic findings in four cases are reported, demonstrating the possible significance of the menstrual dissemination of bits of the uterine mucosa into the venous circulation of the uterus.

CASE 1. Patient aged 32, single. Uterus and right tube and ovary removed March 21, 1925, for a large submucous myoma on the second day of the menstrual period. Pieces of uterine mucosa were found in the blood of a mucosal sinus (Fig. 22), in a receiving sinus of the myometrium (Fig. 24), and also in other veins of the uterine wall (Fig. 25). A mural thrombus containing similar fragments was present in one of these veins (Fig. 26). The "endometrial tissue" in this thrombus stained poorly as compared not only with the uterine mucosa but also with the pieces of the latter found in the lumina of the other vessels of the same specimen (Figs. 27*a* and 27*b*), thus suggesting that they were undergoing degenerative changes and had been separated from the uterine mucosa for a longer time than the latter. There was associated an endometrial cyst of the right ovary which was fused with the posterior layer of the broad ligament, apparently resulting from a previous rupture or perforation of the cyst. The endometrial lining of the cyst showed the same menstrual reaction as that of the uterine cavity. Multiple lesions containing endometrial tissue involved the peritoneum about the right ovary and also were present in the posterior cul-de-sac. The distribution of these lesions was such as to indicate their origin from the escape of the contents of the endometrial cyst of the ovary into the peritoneal cavity.

CASE 2. Patient aged 33, single. Uterus and both tubes and ovaries removed Nov. 20, 1924, three weeks after the last menstrual period, for an extensive peritoneal endometriosis associated with bilateral ovarian cysts of endometrial type. The posterior wall of the uterus was deeply invaded by endometrial tissue apparently developing on its peritoneal surface or at least in the peripheral zone of the uterus. Endometrial emboli were found in four veins of the uterine wall, all of them either arcuate veins or their peripheral branches. The histologic relation of these emboli to the contents of the veins and their lining was such as to lead one to believe that they could not be artefacts (Figs. 28 and 29). Most of the endometrial

tissue in these vessels, both epithelium and stroma, stained poorly as compared with the mucosa lining the uterine cavity, suggesting degenerative changes. I believe that they might have escaped into the venous circulation of the uterus during menstruation. The last flow occurred three weeks before the operation.

CASE 3. Patient aged 53, single. Uterus and both tubes and ovaries removed April 10, 1926, for an endometriosis of its anterior wall, apparently of the direct type (Figs. 14, 15, 16, 17 and 18). Patient had been flowing for five weeks prior to the operation. A gland-like arrangement of "uterine epithelium" was found attached by fibrin to the wall of a receiving sinus (Figs. 32 and 34), thus demonstrating that it must have reached this situation before the tissues had been fixed. In another receiving sinus a polypoid growth of endometrial tissue, apparently covered by endothelium and consisting of a typical uterine gland surrounded by stroma, was attached by a slender pedicle to its wall (Figs. 30, 31 and 33). While an endometriosis of the uterine wall involved the tissues about a branch of this sinus at another level of the block, serial sections showed that the gland of the polyp was not continuous with any endometrial tubule outside of the sinus. (In all cases where I have been able to obtain either serial sections or many sections of polypoid invaginations of endometrial tissues into vessels, it was possible to demonstrate that the apparent gland-like structure in the polyp was but a section of an epithelial tubule continuous with those outside of the vessel (see Figs. 14 to 18 inclusive).) The endometrial polyp in this case must have arisen either from (1) a metaplasia of the endothelial lining of the sinus, (2) an implantation of a fragment of endometrial tissue escaping into the sinus from the uterine mucosa during menstruation, (3) an implantation of a piece of endometrial tissue escaping into a branch of the sinus during the menstrual reaction of the heterotopic endometrial tissue about it, or (4) if it previously had been continuous with the endometrial tissue about a branch of the sinus (at another level), this connection in some way must have been severed.

Another interesting problem presents itself in this case and that is whether or not some of the ectopic endometrial tissue of the uterine wall may have been of metastatic or embolic origin rather than arising from the direct invasion of the myometrium by its mucosa. The uterine epithelium adherent to the wall of a sinus, the polyp

and other findings in the case strongly suggested this possibility (see Figs. 33, 34 and 35).

CASE 4. Patient aged 41, married; one child 14 years old. Uterus, both tubes and ovaries removed March 9, 1926, on the second day of the menstrual period, for a peritoneal endometriosis fusing the anterior surface of the uterus with the bladder, with partial obliteration of the anterior cul-de-sac; a similar lesion obliterating the bottom of the posterior cul-de-sac, with invasion of the sigmoid causing partial intestinal obstruction, and extension downward between the rectum and the vagina and into the posterior vaginal vault. Following the suggestion of Dr. William P. Graves [8] a temporary colostomy was made which later closed spontaneously.

Ovaries were normal and tubes were patent. An endometriosis, in many ways displaying the histologic structure of the direct type, was present in the left half of the posterior uterine wall (Figs. 36, 38 and 39) with but slight invasion of the right half of the posterior uterine wall and that near the fundus. In the right half of the posterior uterine wall endometrial emboli were lying free in the lumina of some of the veins of the peripheral zone (Figs. 37, 43, 53 and 65). Multiple embolic or metastatic growths of endometrial tissue were found in the arcuate veins and also in the peripheral veins of that side (Figs. 37, 49 and 52). Serial sections showed that these deposits of endometrial tissue were attached to the walls or lining of these vessels and were not continuous with any endometrial tissue outside of the vessel. They were evidently due to a localized metaplasia of the endothelial lining of the vessels or an implantation (anchoring) of bits of endometrial tissue similar to those which were found floating about in the lumina of some of them. The study of the specimen suggested that they primarily arose from the menstrual dissemination of fragments of endometrial tissue from the uterine mucosa into receiving sinuses rather than from the menstrual reaction of the ectopic endometrial tissue in the left half of the posterior uterine wall. In only a very few areas of ectopic endometrial tissue of the left half of the posterior uterine wall was there any suggestion of a menstrual reaction (Fig. 40). Had these emboli arisen from this source, one would have expected to have found more of them in the sinuses and veins of that half of the uterus rather than in those of the opposite side. As it was, only a very few were found in the left half of the posterior uterine wall and those in the peripheral zone

(Fig. 41), while many were found in the veins and venous sinuses of the opposite side. A bit of endometrial tissue was found in a sinus of the uterine mucosa (Fig. 55) and also embolic lesions were found in the receiving sinuses of the right half of the posterior uterine wall (Figs. 53 and 54), thus suggesting that it was through such channels that the endometrial tissue had primarily escaped in order to reach the arcuate and peripheral veins of the side in which the embolic lesions were most numerous. It seems reasonable to believe that these endometrial vegetations in the sinuses and veins arose from the anchoring or implantation of endometrial fragments similar to those found floating in these vessels and were primarily derived from the menstrual dissemination of endometrial tissue from the uterine mucosa into the venous circulation of the uterus. On the other hand, some of the endometrial tissue floating in these vessels might be pieces cast off by a menstrual reaction in the embolic vegetations of endometrium growing on their walls.

A peritoneal endometriosis was present in the anterior cul-de-sac fusing the anterior surface of the uterus with the anterior layer of the broad ligament and the peritoneum covering the bladder and apparently arising by peritoneal implantation (Fig. 56). Only the peripheral zone of the anterior uterine wall was invaded by this tissue and no deeper than the corresponding invasion of the anterior layer of the broad ligament and the peritoneum covering the bladder. The entire uterine wall was cut into blocks and many sections were studied from each block. Endometrial tissue was not found in the deeper portions of the anterior uterine wall. Could the endometriosis in this situation possibly have arisen from metastasis to the peripheral vessels of the anterior uterine wall, similar to that already described, and later extending to the peritoneal surface? Such an origin cannot be excluded. Had it been metastatic through veins or lymphatics we would expect to find in it emboli similar to those of the right half of the posterior uterine wall but none was found. In a few places, however, a possible communication of the endometrial tissue with the lumen of a sinus was found where portions of the endometrial tissue were definitely intra- and not retro-endothelial, thus suggesting a possible metastatic origin. It is also possible that the extensive endometriosis of the radial zone of the left half of the posterior uterine wall was of metastatic rather than of direct origin as in only one place was an invasion of the uterine

wall by its mucosa found and that apparently for only a short distance. Its continuation with the extensive endometrial lesions of that wall was not definitely established. The distribution of the endometrial tissue in this portion of the posterior uterine wall (Fig. 36) conformed with the distribution of the bismuth in corresponding sections of the walls of uteri in which the veins had been injected. The endometrial tissue in this lesion was nearly everywhere of the direct type, *i. e.*, wherever the lumen of a vein or sinus could be seen the endometrial tissue was retro- and not intra-endothelial, except in a few areas (where it possibly communicated with the lumen of a vein) such as were present in the peripheral zone. I believe that a metastatic lesion may possibly become entirely retro-endothelial by the growth of the endothelial lining of the vessel over it, as endothelium grows over a thrombus, and subsequently it may take a retro-endothelial course in its extension and thus be histologically indistinguishable from the lesion of a direct endometriosis. In several of the embolic lesions the endothelium of the vein or sinus had grown over portions of the endometrial implant (see Figs. 41, 50 and 52) so as to suggest that it might completely cover it, although nowhere was this conclusively shown.

The posterior surface of the body of the uterus was not adherent but the bottom of the posterior cul-de-sac was occluded by an extensive endometriosis fusing the posterior wall of the cervix with the recto-sigmoid. The nature of the intestinal lesion was not ascertained as the sigmoid was not resected.

The endometriosis of the portion of the posterior vaginal wall, which was excised, was as interesting as that of the uterus. Multiple ectopic endometrial cavities were found filled with blood in which were floating bits of endometrial tissue (Figs. 57, 58 and 60). Similar fragments were found lying free in the lumina of nearby veins and also anchored to or implanted on the walls of veins (Figs. 58, 59 and 66). The actual escape into a vein of the menstrual contents of two of these endometrial cavities could be demonstrated (Figs. 60 and 61). Here again we have evidence that endometrial tissue may be disseminated into veins during menstruation and actually become implanted on the walls of veins, thus demonstrating that the fragments of endometrium set free by the menstrual reaction are sometimes alive and capable of becoming implanted under favorable conditions.

What was the origin of the ectopic endometrial tissue in this case? I have proved that some of the lesions were of mestastatic or embolic origin and also was able to find a direct invasion of the uterine wall by its mucosa. It was not possible, however, to determine whether or not the latter gave rise to all of the endometrial tissue in the extensive endometriosis of that portion of the uterine wall. It was also shown that there were peritoneal lesions of implantation type, possibly due to the escape of menstrual blood through the tubes into the peritoneal cavity. The tubes were patent and endometrial tissue was not found in the ovaries. If one method for the extension or dissemination of endometrial tissue was primarily responsible for the endometriosis in this case, we must choose the metastatic (embolic), *i. e.*, the menstrual dissemination of bits of the uterine mucosa into the venous circulation of the uterus. Some of the tissue emboli might have become retro-endothelial and in their subsequent extension caused the endometriosis of the radial zone of the left half of the posterior uterine wall in which the distribution of endometrial tissue simulated that of the bismuth in the uterine wall whose veins had been injected and were of the type of a direct endometriosis. Metastases in the subperitoneal veins of the uterine walls might have subsequently extended through to the peritoneal surface causing a peritoneal endometriosis with a later extension to the peritoneum covering the bladder in the anterior cul-de-sac and the sigmoid and recto-vaginal septum in the posterior cul-de-sac. I admit the possibility of the above but am inclined to believe that there was more than one method of origin of the ectopic endometrial tissue in this case.

## The Bearing of these Studies on the Etiology of Other Varieties of Endometriosis than those Just Described

If fragments of endometrial tissue escape into the venous circulation of the uterine wall during menstruation and become implanted on the endothelial surface of the veins and venous sinuses of that organ and if a similar condition arises in veins about misplaced endometrial cavities in the vagina, it is natural to assume that a like implantation of this tissue might occur in veins remote from these endometrial cavities. This might account for the origin of misplaced endometrial tissue at a distance from the uterus, such as

some of those in the vagina, vulva, groin, or even the umbilicus, the latter through the round ligament and epigastric veins.

If menstrual blood carrying with it bits of endometrial tissue escapes into the venous circulation of the uterus and these bits sometimes become implanted on the endothelial surface of the veins and venous sinuses of the uterine wall, and if a like condition arises from the menstrual reaction of the mucosa of ectopic endometrial cavities in the vagina, we might infer that bits of endometrial tissue carried by menstrual blood escaping into the peritoneal cavity from any source (such as a back flow through the tubes from the uterine cavity, from the rupture or perforation of an endometrial cyst of the ovary or the menstrual reaction of endometrial tissue on the surface of the various pelvic structures) might become implanted on the mesothelial surface of the peritoneum and give rise to at least some of the lesions of peritoneal endometriosis. Jacobson has shown by his experimental work in rabbits and monkeys that bits of the uterine mucosa of these animals, scattered in their peritoneal cavities, become implanted on the peritoneum causing a peritoneal endometriosis similar to that found in human beings. The findings in the cases just reported demonstrate that bits of endometrial tissue disseminated by menstruation are sometimes alive and can become implanted on the endothelial lining of veins and venous sinuses. We know that menstrual blood, containing bits of endometrium, at times escapes into the peritoneal cavity from the above mentioned sources. Peritoneal endometriosis in women often occurs in situations and under conditions indicating (or at least suggesting) its origin from these sources.

## The Origin of Endometrial Tissue in Uterine Endometriosis

The study of endometriosis of the uterine wall demonstrates that it may arise in four, and possibly more, ways.

1. The direct invasion of the uterine wall by its mucosa or by tubal mucosa — a direct or primary uterine endometriosis.
2. The invasion of the external portion of the uterine wall by the direct extension of endometrial tissue from an ectopic endometrial focus in the pelvis — an indirect or secondary uterine endometriosis, by extension.

3. From endometrial tissue implanted or developing on its peritoneal surface — an implantation or peritoneal uterine endometriosis.
4. From the menstrual dissemination of endometrial tissue into the venous circulation of the uterus, either from the mucosa lining its cavity or from ectopic endometrial tissue in the myometrium — an embolic or metastatic uterine endometriosis.
5. The possibility of metastasis through the lymphatics, and also of developmental inclusions of the uterine mucosa in the myometrium, must be considered. The origin of endometrial tissue from a metaplasia of the endothelial lining of vessels does not appeal to me.

## Summary

A histologic study was made of sections of uteri removed during the various stages of the menstrual cycle, in which the veins had been injected with bismuth. By this means it was demonstrated that there are venous capillaries and large venous sinuses in the uterine mucosa and that the latter empty into similar sinuses (receiving) in the uterine wall. During menstruation, blood escapes from the mucosal vessels into the surrounding tissues, and bits of the mucosa are often set free in the extravasated blood. These studies suggest that this menstrual blood containing fragments of endometrial tissue, at times escapes through a ruptured mucosal sinus into the venous circulation of the uterus.

Sections of misplaced endometrial tissue, wherever situated and irrespective of its origin, also suggest that a like dissemination of fragments of this tissue occurs during menstruation.

In menstruating uteri bits of the uterine mucosa at times actually escape into the venous circulation of the uterus through these channels. I have not been able definitely to prove this in a direct endometriosis of the uterine wall but believe that it also must occur in this condition. The escape into veins of the contents of two ectopic endometrial cavities was found in an endometriosis of the posterior vaginal wall.

In one uterus removed during menstruation (Case 4) in which bits of endometrial tissue were found in the blood in veins and venous sinuses of the uterine wall, multiple embolic or metastatic-like growths of endometrial tissue also were present in these vessels.

By serial sections it was shown that these growths either arose from or were implanted on the walls or linings of these vessels and did not arise from the invasion of the latter by endometrial tissue from without. These embolus-like growths of endometrial tissue must have originated either from a localized metaplasia of the endothelial lining of the veins and venous sinuses or else from the actual anchoring and implantation of endometrial tissue similar to that found free in some of the vessels of the specimen. The study of the entire uterus demonstrated that, while some of the endometrial emboli lying free in the vessels of the uterine wall might have arisen from the menstruation of ectopic endometrial tissue in that organ, the latter primarily were derived from the mucosa lining the uterine cavity. In the endometriosis of the posterior vaginal wall of this case, similar endometrial emboli and embolic vegetations of endometrial tissue were present there in veins about misplaced endometrial cavities and the actual escape of the menstrual contents of two of these cavities into a vein was seen.

In a second uterus, also removed while the patient was flowing (Case 3), somewhat similar lesions were found in which their embolic origin was not as definitely established as in Case 4. Nevertheless I believe that they had a similar origin.

If these observations are correctly interpreted, they show that bits of endometrial tissue disseminated by menstruation from the mucosa lining the uterine cavity and also from ectopic endometrial foci, are not always dead but are sometimes alive and are capable of becoming implanted on the endothelial surface of nearby veins and venous sinuses.

They further suggest that bits of endometrial tissue carried by menstrual blood into the venous circulation might cause metastatic growths of endometrial tissue at a distance from the original focus, and also that similar fragments of endometrial tissue carried by menstrual blood escaping from any source into the peritoneal cavity at times might cause the lesions of peritoneal endometriosis.

## Conclusions

1. Fragments of endometrial tissue, at times, are disseminated into the venous circulation during menstruation, from the mucosa lining the uterine cavity and also from ectopic endometrial foci.

2. Metastatic or embolic endometriosis arises from the implantation of these emboli in nearby veins.

3. Endometrial tissue set free by menstruation, therefore, is sometimes not only alive but may actually continue to grow if transferred to situations favorable to its existence.

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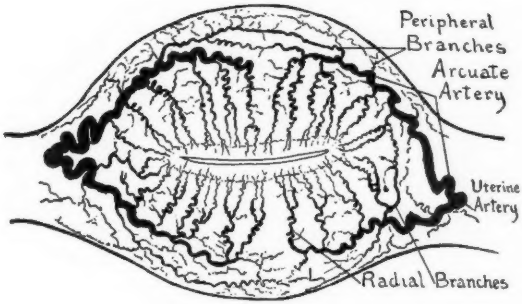
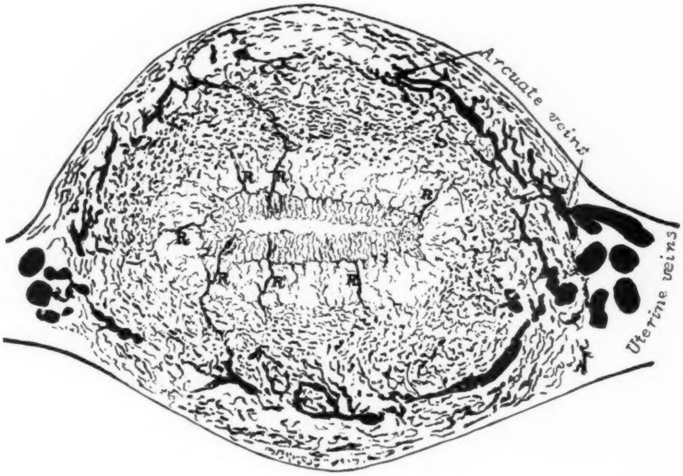
PLATE 20

FIG. 1. The general plan of the distribution of the intrinsic uterine arteries as seen in a cross-section of the body of the uterus. Composite tracing (slightly enlarged) of roentgenograms of thin cross slices of the uterus; arteries injected with bismuth. The arcuate arteries, which arise in pairs from each uterine artery, divide the uterine wall into a narrow outer or peripheral zone nourished by the peripheral branches of these arteries and a wide inner or radial zone supplied by their radial branches. The latter terminate in the mucosa. The greater portion of the arterial supply of the uterus is directed toward its mucosa.

FIG. 2. The general plan of the venous outlets of the uterine tissues as seen in a cross-section of the body of the uterus. Tracing (enlarged and with some of the receiving sinuses accentuated) of a roentgenogram of a thin cross slice of the uterus, veins injected with bismuth; hyperemia due to tubal pregnancy. The venous blood is conveyed from the uterine tissues mainly by the arcuate veins which empty into the uterine plexus of veins situated between the layers of the broad ligament. The arcuate veins receive blood from both the peripheral and the radial zones of the uterus. The venous capillaries of the mucosa, some of which are dilated forming sinuses (Fig. 3), empty into the venous sinuses of the radial zone of the myometrium and these in turn into the arcuate veins. Some of these sinuses are relatively large and radiate from the endometrium (R of illustration). It is obvious that foreign material gaining access to the lumina of the mucosal sinuses might escape through the radial or receiving sinuses (R) into the deeper sinuses and veins of the uterine wall.


Peripheral
Branches
Arcuate
Artery
Uterine
Artery
Radial Branches

1


Arcuate veins
Uterine veins

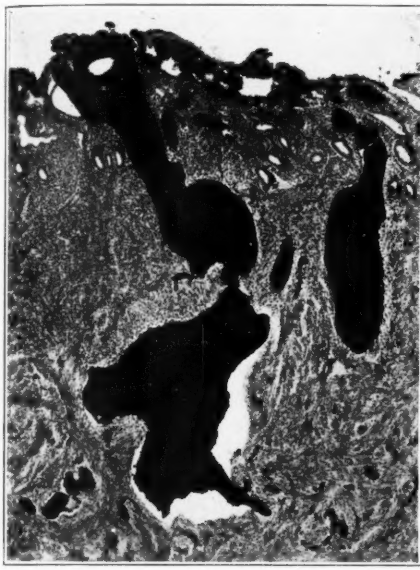
2

PLATE 21

FIG. 3. Photomicrograph ( $\times$ 25) of a section of the uterine mucosa and underlying muscularis, veins injected with bismuth; from a patient with tubal pregnancy. Condition present corresponds with that found at the close of the menstrual period. Two receiving sinuses are shown, one of which extends almost to the surface of the uterine mucosa. The portion of the sinus situated within the mucosa might be designated a mucosal sinus. These sinuses are but spaces lined by endothelium and without definite walls. In the non-injected specimen they are often empty or contain very little blood and therefore could readily be mistaken for lymph vessels. Any foreign material in the lumen of the mucosal sinus might easily escape into the receiving sinus of the muscularis with which it is continuous.

FIG. 4. Photomicrograph ( $\times$ 25) of a section of the uterine mucosa and underlying muscularis, veins injected with bismuth; interval stage of the menstrual cycle. The mucosa is about twice as thick as that shown in Fig 3. A receiving sinus is present and due to the postmenstrual growth of the mucosa, the sinus is more deeply situated than those shown in Fig. 3.

FIG. 5. Photomicrograph ( $\times$ 10) of a section of the uterine mucosa and underlying muscularis in the angle between the anterior and posterior uterine wall, veins injected with bismuth; premenstrual stage of the menstrual cycle. The mucosa is much thicker than that shown in Fig. 4 (less than half the magnification). Several dilated capillaries or mucosal sinuses are present. As the principal menstrual reaction usually occurs in the superficial portion of the mucosa, could bits of the latter escape into these sinuses during menstruation?



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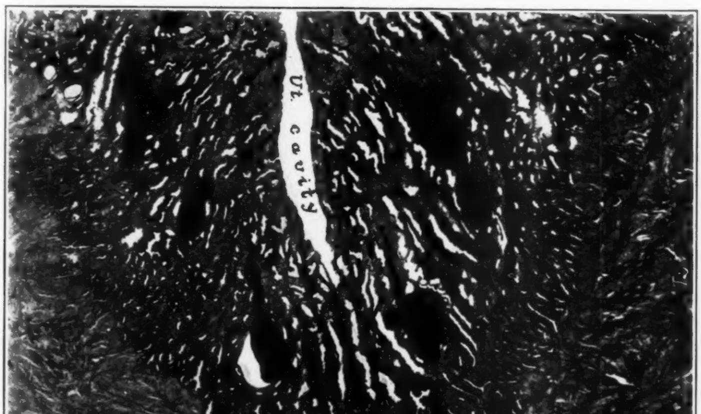


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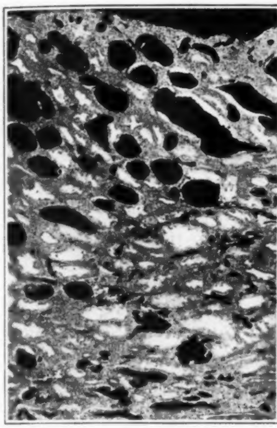
## Plate 22

Figs. 6, 7 and 8. Three photomicrographs ($\times$ 20) of sections of the uterine mucosa and underlying muscularis, veins injected with bismuth; patient menstruating. All sections from the same uterus but from different portions and showing different stages in the menstrual reaction. The photomicrograph to the left shows the dilated mucosal capillaries, the middle one the rupture of the same, due to the menstrual reaction, thus permitting the injection mass to escape into the tissues of the mucosa. Fig. 7 represents a still later stage of the menstrual reaction. The superficial layer of the uterine mucosa has been separated from the deeper layer by the extravasated injection mass and bits of the uterine mucosa lie free in this mass just as they are found in the extravasated blood of the non-injected menstruating endometrium. Could menstrual blood carrying with it bits of the mucosa escape into the ruptured dilated venous capillaries and from these into the venous circulation of the uterus?

Fig. 9. Photomicrograph ($\times$ 25) of a section of the uterine mucosa and underlying muscularis, veins injected with bismuth; end of the first day of the menstrual period. A mucosal sinus is shown with rupture of its endothelial lining permitting the injection mass to escape into the tissues of the mucosa. It is conceivable that fragments of the uterine mucosa set free by the extravasated blood, might at times escape into the lumen of such a sinus and be carried with that blood into the venous circulation of the uterus (see Fig. 10).

Fig. 10. Photomicrograph ($\times$ 5) of a cross-section of the uterine wall including its mucosa (section from which the photomicrograph shown in Fig. 9 was made). The mucosal sinus (M.S.) of the endometrium, which has ruptured, is shown and also a receiving sinus which carries the venous blood from the mucosa. (It is not evident that the mucosal sinus in this photomicrograph empties into this receiving sinus but either it does, or else into one like it.) Blood carrying with it any material, such as bits of the uterine mucosa, which had escaped into the lumen of a mucosal sinus, might readily be carried through a receiving sinus into any of the venous sinuses of the uterine wall and especially into the arcuate veins as the main blood stream is from the mucosa through the receiving sinuses into these veins.



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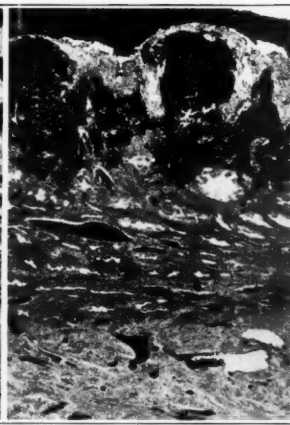

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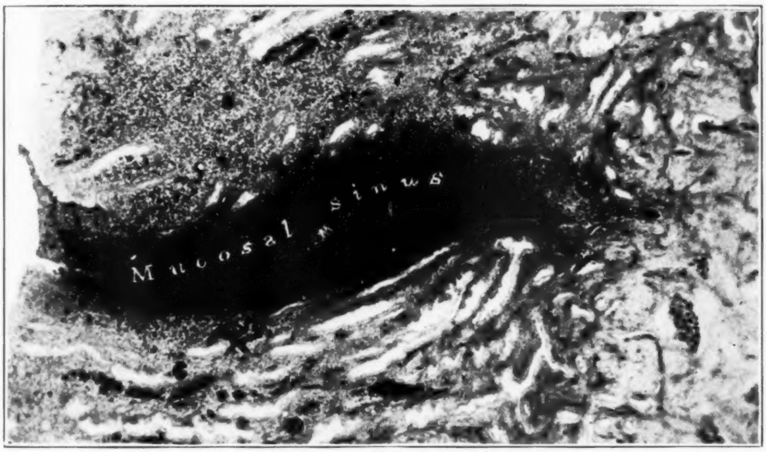


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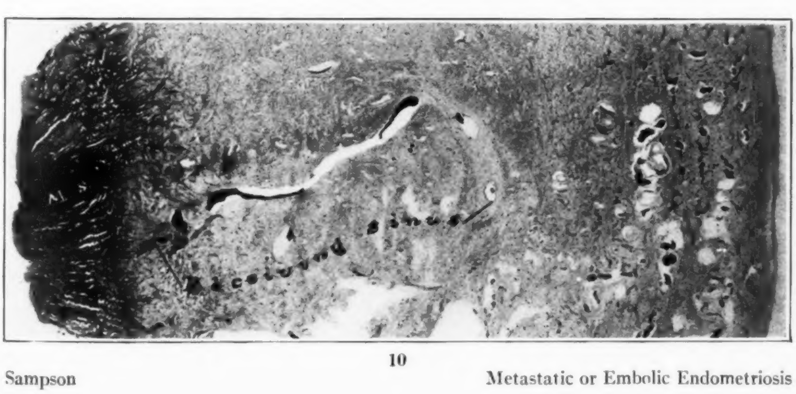

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## Plate 23

Fig. 11. Photomicrograph ($\times$ 25) of a cross-section of the peripheral zone of the uterine wall, veins injected with bismuth; premenstrual stage of the menstrual cycle. The veins and venous sinuses in this section are but spaces, between the muscle bundles, lined by endothelium. They empty into the arcuate veins, bottom of the photomicrograph. (Some of the injection mass has fallen out of the larger vessels.) In the non-injected specimen these vessels are often empty or contain very little blood and may readily be taken for lymphatics. There are no valves in the veins and venous sinuses of the uterine wall. The various physiologic changes in the venous pressure in these vessels, due to uterine relaxation and contraction, might force foreign material suspended in the blood into any of the veins and sinuses of the uterine wall including those of the peripheral zone, since they are to one side of the main blood stream from the mucosa to the arcuate veins, and thence to the uterine veins. For physiologic reasons we might expect to find bits of the uterine mucosa, escaping into the venous circulation, in the vessels of the peripheral zone, even in the small vessels near the serosa where they might be retained.

Fig. 12. Photomicrograph ($\times$ 25) of a section of the uterine wall with a direct or primary endometriosis (adenomyoma due to the invasion of the uterine wall by its mucosa), veins injected with bismuth. Dilated venous capillaries (sinuses) are present in this misplaced uterine mucosa similar to those of the mucosa lining the uterine cavity. In the menstrual reaction of misplaced endometrial tissue, fragments of it are set free in extravasated blood just as they are set free in the extravasated blood of the menstruating mucosa lining the uterine cavity. Could some of these bits gain access to the lumina of its venous sinuses and be carried into the venous circulation of the uterine wall? I have examined many sections of primary endometriosis in uteri removed during menstruation, and have not been able to prove that this occurs. I believe that it must occur and will be definitely proved.

Fig. 13. Photomicrograph ($\times$ 25) of a section of the uterine wall with an endometriosis near its serosa; veins injected with bismuth. The patient had bilateral ovarian hematomas of endometrial type associated with a peritoneal endometriosis. The misplaced endometrial tissue in this section also contains dilated venous capillaries or sinuses. In the menstrual reaction of this misplaced "uterine mucosa" bits of the latter might also gain access to the lumina of its sinuses.

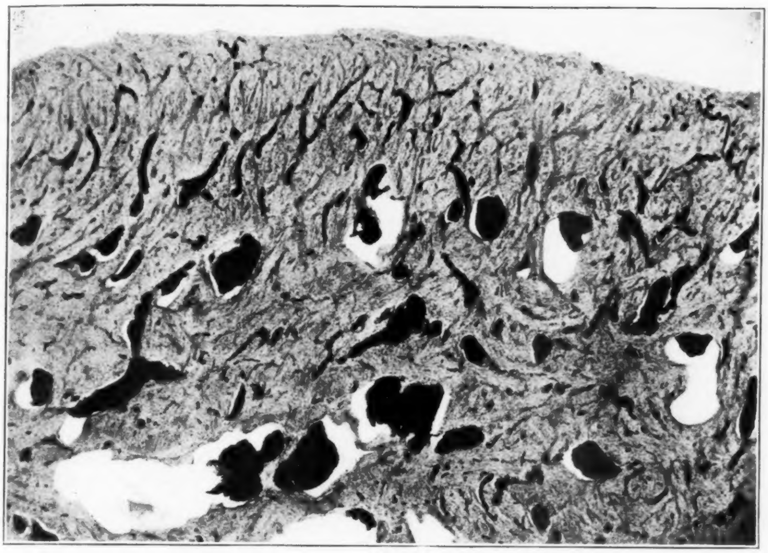

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PLATE 24

FIGS. 14 and 15. Two photomicrographs ( × 60) of sections from a series showing the invagination of the endometrial tissue of a direct or primary endometriosis into the lumen of a receiving sinus of the uterine wall (Case 3). The first section shows the relation of the endometrial tubule (E.T.) to a receiving sinus (the latter is not a lymph vessel as it contains a small amount of blood and corresponds in its structure and situation with the receiving sinuses of injected specimens). In the second photomicrograph the same endometrial tubule surrounded by stroma is shown bulging into the lumen of the sinus but covered by the endothelial lining.

FIGS. 16, 17 and 18. Three photomicrographs ( × 60) of sections from the same series as those shown in Figs. 14 and 15. The first shows a greater bulging of the tubule (E.T.) into the sinus (compare with Fig. 15). In the second one the tubule lies apparently almost entirely within the lumen of the sinus and without any evidence of the endometrial tissue from which it came. The third photomicrograph shows a cross-section of the tubule surrounded by stroma and covered by endothelium and attached to wall of the sinus by the latter. The tubule surrounded by its stroma is apparently within the lumen of the vessel but is extra- or retro-endothelial and not truly intravascular; its epithelium and stroma are directly continuous with the tubule shown in Figs. 14 and 15. The latter was possibly continuous with a tubule of the mucosa lining the uterine cavity. The endometrial tissue within the sinus is not of metastatic or embolic origin because serial sections showed that both the epithelium and stroma are continuous with similar endometrial tissue outside of the sinus (compare with Figs. 30, 31 and 33 from the same case).


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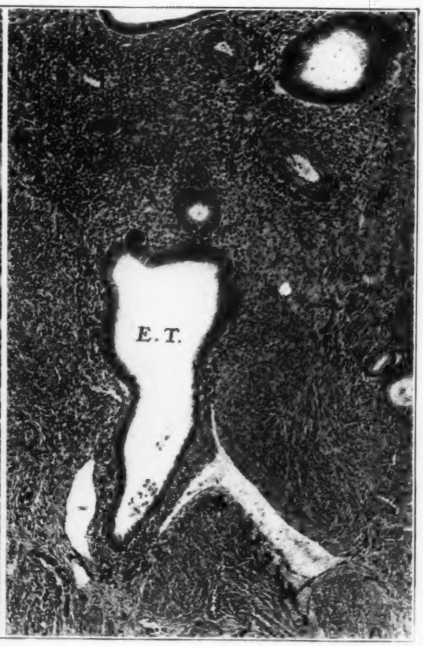


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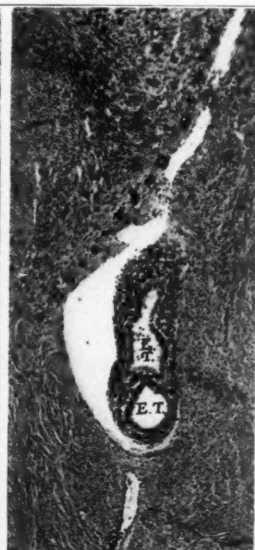


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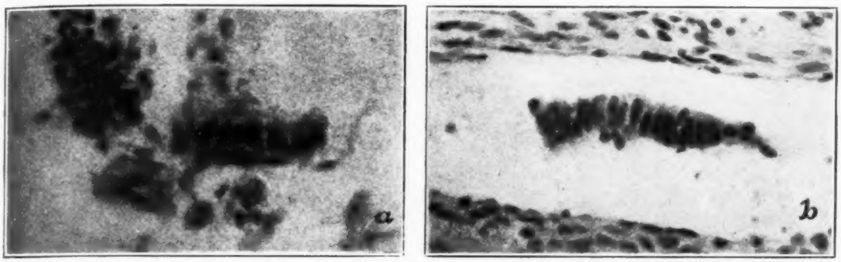
18

FIG. 19*a*. Photomicrograph (× 310) of the parametrium with bits of the uterine mucosa (stroma and epithelium) enmeshed in the tissues of the former. The block of the uterine wall from which the section was made included a portion of the uterine mucosa (menstruating). Bits of the friable uterine mucosa were probably set free in the embedding solutions and some of these became entangled in the loose tissues of the parametrium. Similar pieces of the uterine mucosa, in like manner, might fall into a gaping uterine sinus or vein (see Figs. 19*b*, 20 and 21).

FIG. 19*b*. Photomicrograph (× 310) of a receiving sinus of a uterine wall showing a strip of "mucosal epithelium" lying free in its lumen; patient menstruating at the time of the operation. The block of the uterus from which the section was made included a portion of the uterine mucosa. Serial sections had been made and the epithelium was not attached to the wall of the sinus. While I cannot exclude its origin from the menstrual dissemination of this bit of the uterine mucosa into the sinus, I believe that it is more apt to be an artefact and that it arose in the same manner as the fragments of mucosa shown in Fig. 19*a*. Had the epithelium gained access to the sinus before the specimen had been fixed I would expect to find it attached to the wall of the sinus or else with blood about it (see Figs. 25, 27 and 53).

FIG. 20. Photomicrograph (× 310) of a portion of a vein of the uterine wall showing a piece of the uterine mucosa lying free in its lumen; from the same uterus as that shown in Fig. 19*a*; section taken near the surface of another block. I cannot exclude the origin of this tissue from the menstrual dissemination of bits of the uterine mucosa into the venous circulation of the uterus, but for the reasons given in the legend of Fig. 19*b* I believe that it also is more apt to be an artefact.

FIG. 21. Photomicrograph (× 60) of a section of an arcuate vein containing both blood and a bit of the uterine mucosa, the latter lying free in the lumen of the vein, from the same uterus as those shown in Figs. 19*a* and 20 but from a different block of the uterus and from a section taken near the middle of the block. The lumen of the vein is over half filled with blood but the tissue lies free in the empty portion of the vein. It is much more difficult to decide the origin of this bit of tissue. I believe that it also is more apt to be an artefact. A piece of uterine mucosa could drop into the lumen of the unfilled portion of a vein as easily as into an empty one. Had the tissue been embolic from the menstrual reaction it would probably either be partially or wholly embedded in the blood or attached to the lining of the vessels as those shown in Figs. 34, 43 and 53.

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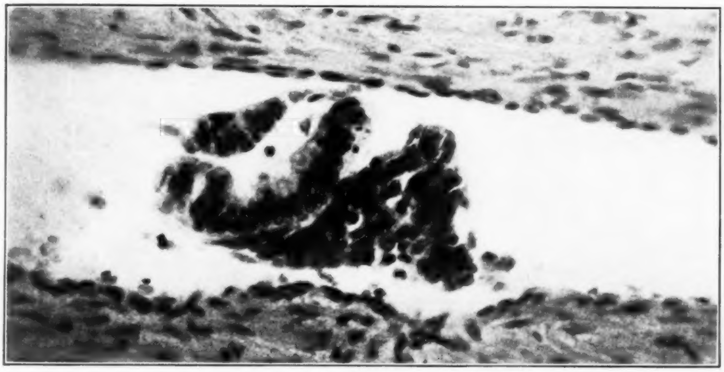

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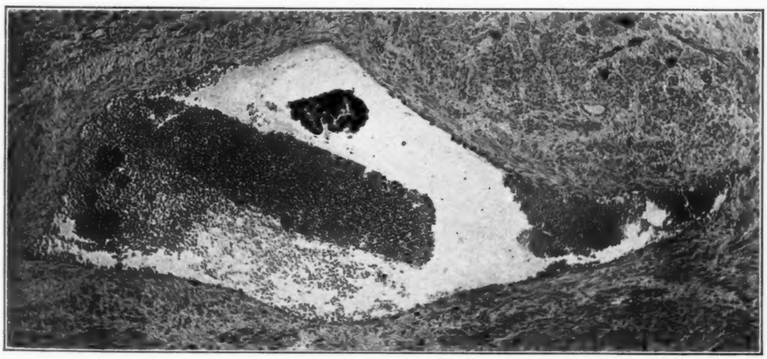

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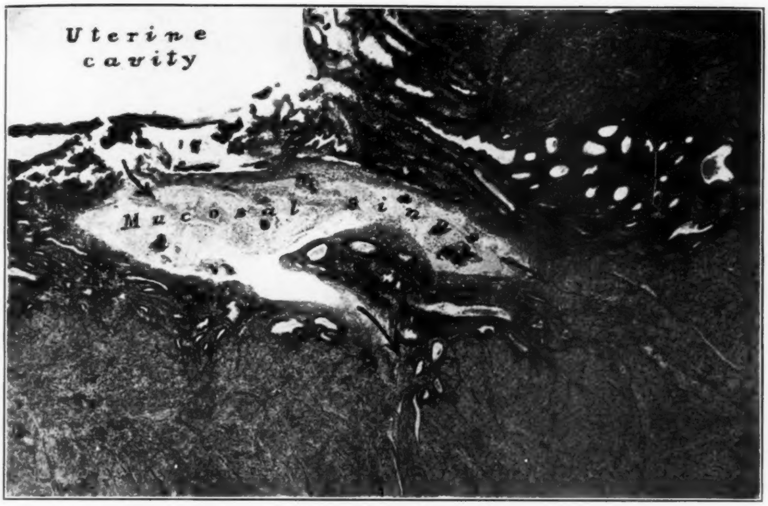
## PLATE 26

FIG. 22. Photomicrograph ($\times$ 25) of the uterine mucosa and underlying muscularis (in the angle between the anterior and posterior uterine wall) showing blood and bits of the uterine mucosa in a large mucosal sinus; second day of the menstrual period (Case 1). The mucosal sinus is unusually large as though two sinuses had become fused. The mucosa over the sinus shows a characteristic reaction of menstruation with the separation of its superficial from its deeper layers by the extravasation of blood into the tissues of the mucosa (Fig. 7) and evidence that blood containing bits of the latter had escaped into the uterine cavity. There is just as strong evidence that some of this extravasated menstrual blood, carrying with it bits of the uterine mucosa, had escaped through the ruptured mucosal sinus into the lumen of the latter (see upper arrow). It is also possible that fragments of the uterine mucosa might be carried from the mucosal sinus into the receiving sinuses of the myometrium and thence into the deeper vessels of the uterine wall (see Figs. 23, 24 and 25). Only the beginning of the receiving sinuses of the myometrium, into which this mucosal sinus empties, appears in this section (see the two lower arrows).

FIG. 23*a*. Photomicrograph ($\times$ 25) showing a mucosal sinus undoubtedly emptying into a receiving sinus. It is a venous sinus and not a lymph vessel because the veins of the uterus had been injected with bismuth.

FIG. 23*b*. Photomicrograph ($\times$ 25) of the uterine mucosa and underlying muscularis from a section close to the one shown in Fig. 22. A vessel with the same structure, situation and general course as that shown in Fig. 23*a* is present and I therefore believe that it also is a venous sinus and not a lymph vessel. This sinus contains a small amount of blood and bits of endometrium (end) in both its mucosal and myometrial portions similar to those shown in the mucosal sinus of Fig. 22. I believe that it is possibly the receiving sinus into which the mucosal sinus, shown in Fig. 22, emptied. It is situated where the latter should be. (Unfortunately, serial sections had not been made. Many sections had been cut from the block and not all of them saved before the condition shown in Fig. 22 had been seen.)

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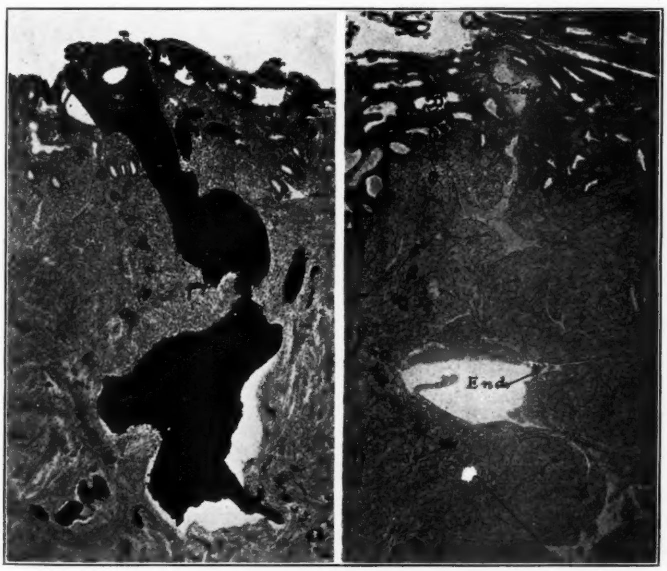


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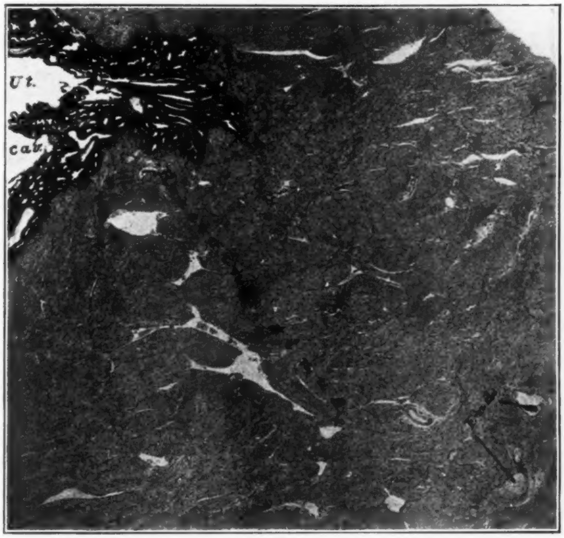
PLATE 27

FIG. 24. Photomicrograph ($\times$ 10) of a section from the same block of the uterine wall as those shown in Figs. 22 and 23. A receiving sinus is present radiating from the uterine mucosa (the same one shown in Fig. 23*b*). It contains a small amount of blood in places and also small bits of the uterine mucosa. Larger bits of the uterine mucosa are present in the vessels indicated by the pointer "End." (see Figs. 25 and 27). Serial sections were not made and therefore it cannot be proved that this receiving sinus emptied into the vessels containing the larger bits of the uterine mucosa but it probably did.

FIG. 25. Photomicrograph ($\times$ 60) of the two vessels containing bits of the uterine mucosa indicated by the pointer "End." of Fig. 24. Other sections demonstrate that these are but two sections of the same vessel. They contain bits of uterine mucosa surrounded by blood and the latter is adherent to the lining of the vessels, thus demonstrating that these fragments gained access to the lumen of the vessel before the tissues were fixed and are not artefacts as those shown in Figs. 20 and 21. The tissue in these vessels has the same histologic structure as that in the extravasated blood of the mucosa lining the uterine cavity, in the mucosal sinus and that in the receiving sinus. I believe that it reached its present situation by menstrual dissemination into the venous circulation of the uterus through the channels indicated in Figs. 22, 23 and 24.

FIG. 26. Photomicrograph ($\times$ 60) of a section of the uterine wall showing a mural thrombus attached to the lining of a vessel from the same block of the uterus as the section shown in Fig. 25. This vessel was situated a little deeper in the uterine wall than that shown in Fig. 25. For a higher magnification showing its histologic structure, see Fig. 27*b*.

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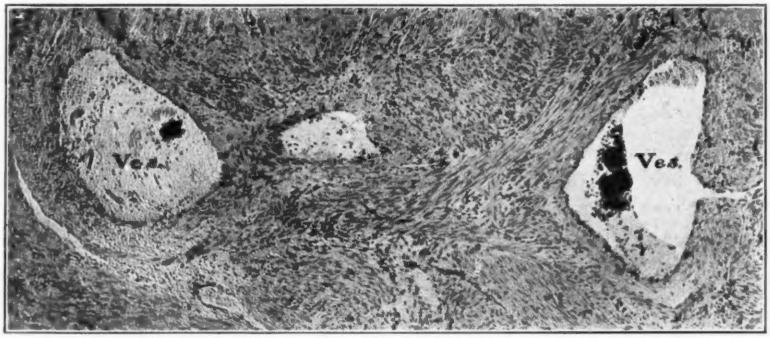


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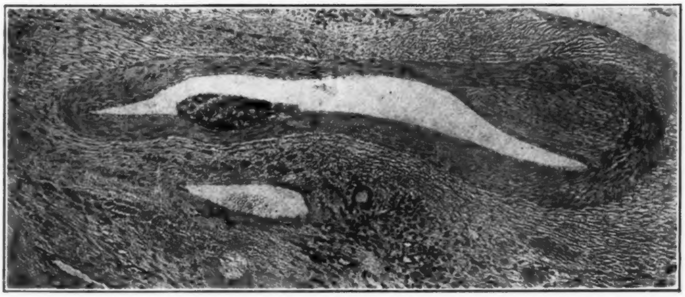

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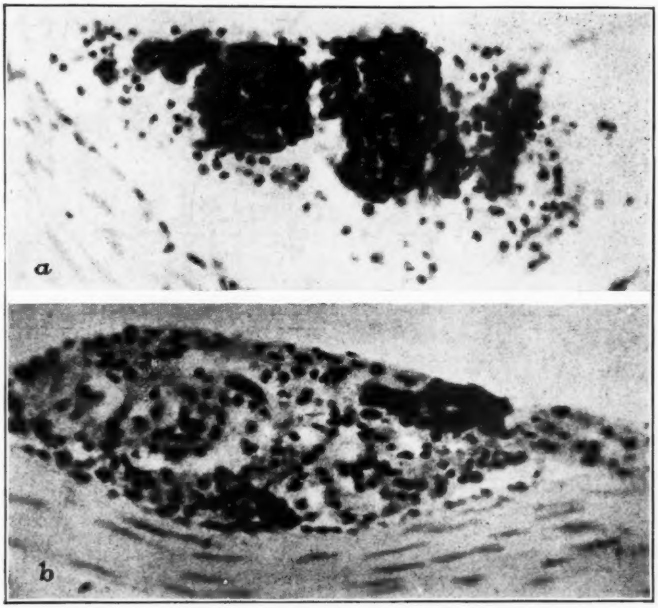
PLATE 28

FIG. 27*a*. Photomicrograph ($\times$ 310) of the contents of the vessel containing the larger amount of endometrial tissue shown in Fig. 25. It consists of stroma and epithelium in a fair state of preservation and identical in their structure with that of similar bits of endometrial tissue in the extravasated blood of the uterine mucosa and in the mucosal sinus of the section shown in Fig. 22.

FIG. 27*b*. Photomicrograph ($\times$ 310) of the mural thrombus shown in Fig. 26. The thrombus is attached to the endothelial lining of the vessel. It consists of fibrin, leucocytes, epithelium-like cells and two clumps of cells which might be interpreted as degenerating stromal cells. It would seem to represent a later stage of the condition shown in Fig. 27*a* and might indicate that the "endometrial tissue" in the thrombus is either dead or at least in a poor state of preservation.

FIG. 28. Photomicrograph ($\times$ 310) of a portion of a peripheral vein in the fundus of the uterus showing blood and bits of "uterine mucosa" attached to the lining of the vessel (Case 2). The block from which the section was made was cut from the uterus after the latter had been hardened in formalin for a few days. The smaller fragment is adherent to the lining of the vessel while the larger is partially enveloped in blood and fixed to the wall of the vessel, thus demonstrating that they must have reached their present situation before the tissues of the specimen had become fixed. The last menstrual period occurred three weeks before the operation. More sections were made from the same block and a similar condition was found in another vein (see also Fig. 29).

FIG. 29. Photomicrograph ($\times$ 310) of a portion of an arcuate vein of the anterior uterine wall from the same uterus as the section shown in Fig. 28 but from a block taken from the uterus at a later date. Over a year after the operation many blocks were taken from the uterus and many sections were studied from each block, and in two vessels of the uterine wall embolic endometrial tissue was found, such as is shown here. The endometrial tissue is adherent to the lining of the vessel and the former stains poorly compared with the mucosa lining the uterine cavity (in the same section) thus suggesting that the former had undergone degenerative changes. The findings in these two cases demonstrate that bits of the uterine mucosa escape into the venous circulation of the uterus during menstruation and suggest that they may be retained in the sinuses of the uterine wall. They also suggest that this tissue may not always live.

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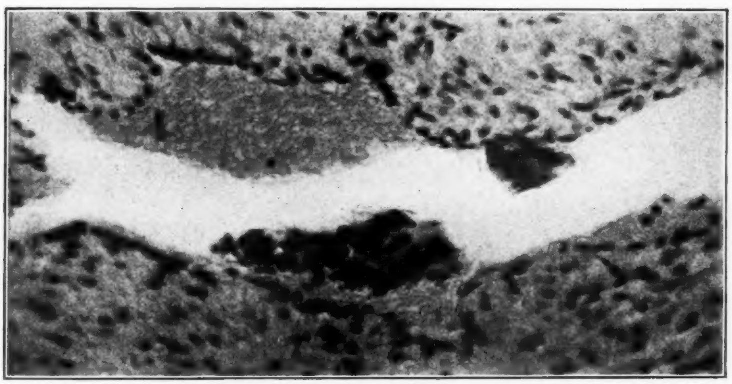

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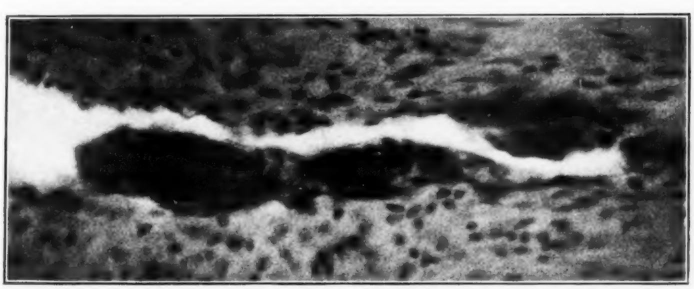

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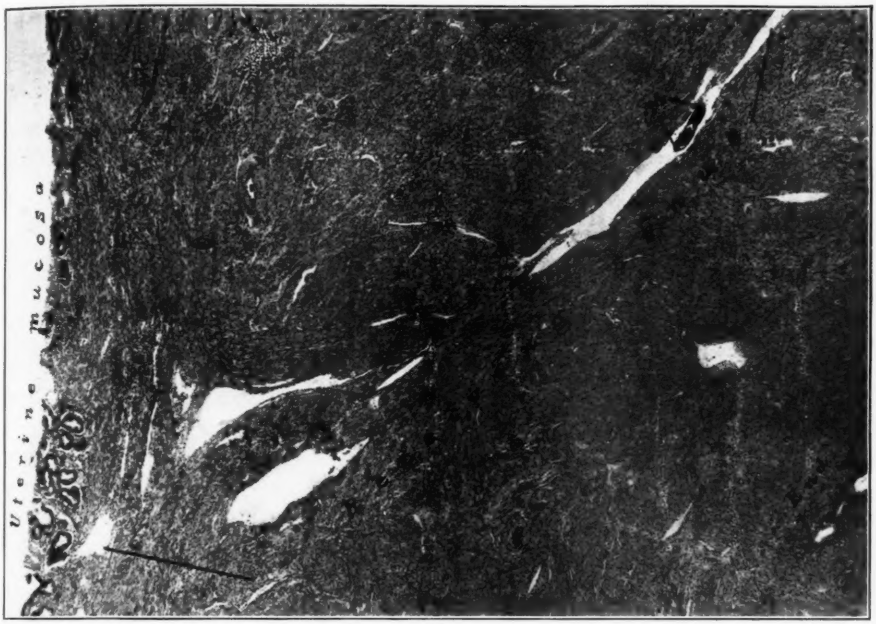
## Plate 29

Fig. 30. Photomicrograph ( × 20) of a section of the uterine wall, including its mucosa, demonstrating an embolus-like piece of the latter (M) in a receiving sinus (Case 3). Serial sections had been made of this portion of the block and it was shown that this receiving sinus extended into the mucosa and that the "embolus had lodged" at the site of a branching of the sinus. An endometriosis (End.) is present in this section of the uterine wall which, from an extra-endothelial position, bulged into a branch of the receiving sinus, as shown by sections of the block taken at another level and similar to the lesion shown in Fig. 14.

Fig. 31. Five photomicrographs ( × 60) of sections showing the appearance of the endometrial tissue in the receiving sinus of Fig. 30, at different levels of the series. *a* shows a bit of stroma apparently adherent to the lining of the sinus but really not attached. *b* (from the same section shown in Fig. 30) demonstrates epithelium arranged in the form of a gland and surrounded by stroma. This tissue apparently lies free in the lumen of the sinus at this level as does also the tissue shown in *c*. *d* demonstrates that the bit is really a polyp, attached by a slender pedicle to the wall of the sinus (for a higher magnification of this polyp see Fig. 33). *e* shows the last appearance of the epithelium in the polyp with a fragment of stroma to the left of it. The series of sections from which these were chosen demonstrated that an endometrial polyp was present in this sinus attached by a pedicle to the wall of the latter and apparently surrounded by endothelium just as similar polyps arise from the invasion of endometrial tissue into a vessel pushing the endothelium ahead of it (see Figs. 14 to 18 inclusive, from the same case) but differing from the latter in that it was conclusively shown that the gland lay entirely within the sinus and was not a cross-section of a tubule continuous with a similar structure outside of the vessel.

Fig. 32. Four photomicrographs ( × 60) of sections showing embolic "uterine" epithelium attached by fibrin to the wall of a receiving sinus. From the same block of the uterine wall as the sections shown in the preceding illustrations but at a different level and in another portion of the block. Serial sections demonstrated that the epithelium, arranged in the form of a gland, lay entirely within the sinus, was not covered by endothelium and was not continuous with any endometrial tissue outside of the sinus. It is attached to the wall of the sinus by fibrin, thus demonstrating that it reached its present situation before the tissues had been fixed, either from the trauma of the operation, the incision of the uterus immediately after the operation or the menstrual dissemination of this tissue into the sinus (patient flowing at the time of the operation). For a higher magnification of the lesion in *c* see Fig. 34.

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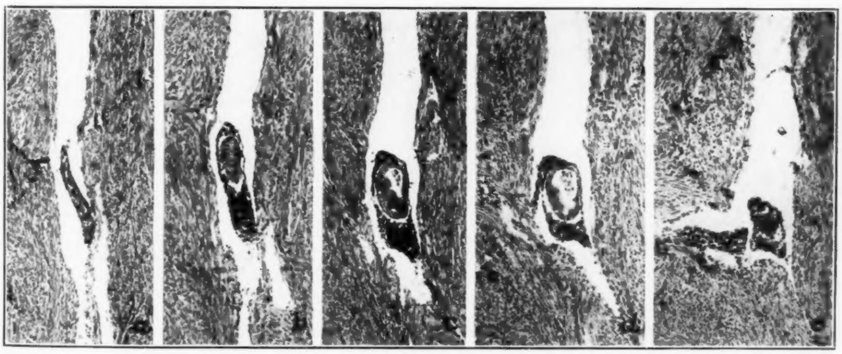

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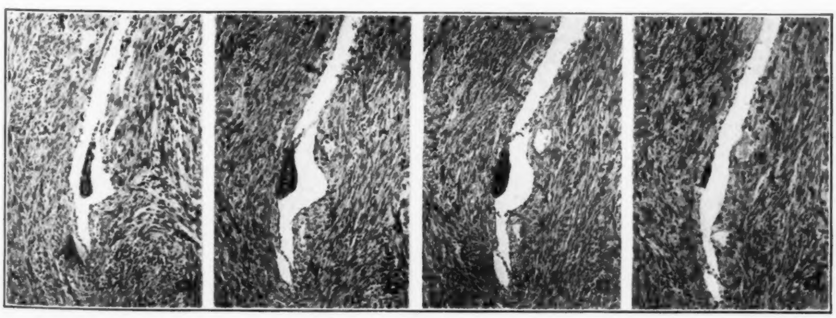

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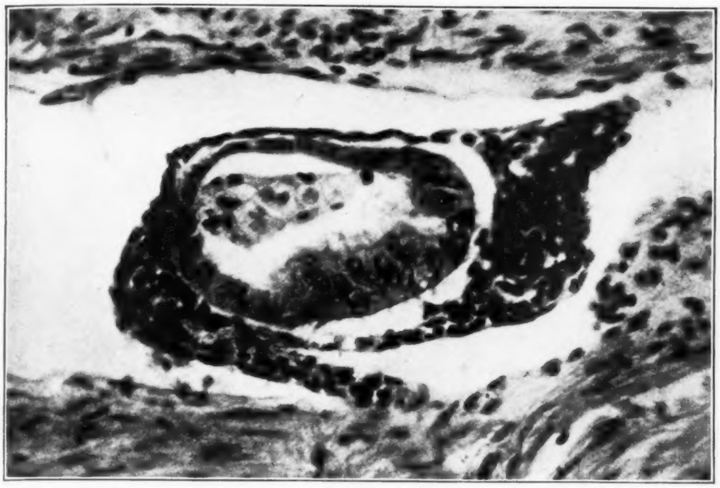
## Plate 30

Fig. 33. Photomicrograph (× 310) of the endometrial polyp shown in *d* of Fig. 31. It consists of an epithelial gland surrounded by endometrial stroma and the latter apparently covered by endothelium; it is extra- or retro-endothelial and is attached to the wall of the sinus by a slender pedicle. This endometrial polyp must have arisen either from a metaplasia of the endothelial lining of the sinus, an implantation of a bit of the uterine mucosa escaping into the sinus during menstruation (Fig. 31), an implantation from the menstrual reaction of the heterotopic endometrial tissue bulging into a branch of the sinus (see Fig. 31) but separated from the lumen of the sinus by its endothelial lining, or if it previously had been continuous with the latter this connection in some way must have been severed. For a possible explanation of its origin see Fig. 34.

Fig. 34. Photomicrograph (× 310) of the "uterine" epithelium attached to the wall of the sinus by fibrin (Fig. 32*c*). The epithelium is arranged in the form of a gland and is entirely intravascular. Its possible origin was discussed in the legend of Fig. 32. If it is a menstrual embolus, as well it might be, the origin of the polyp shown in Fig. 33 can readily be explained. Should the endothelium of the sinus cover such an implant and the endometrial tissue live, it might readily develop into a polyp similar to that shown in Fig. 33 or a lesion similar to that shown in Fig. 35.

Fig. 35. Photomicrograph (× 310) of a portion of a sinus, a branch of the sinus shown in Figs. 30 and 31, with extra-endothelial endometrial tissue bulging into its lumen. Serial sections demonstrated that this endometrial tubule was not continuous with the gland in the polyp shown in Fig. 33. The series beyond this portion of the block was incomplete and the relation of this endometrial tissue to that outside of the sinus was not determined. I believe that it might have been continuous with the latter. If true, the latter might have arisen from this and not the reverse. The conditions shown in Figs. 33 and 34 suggest that this endometrial tissue might have developed from the implantation of embolic endometrial tissue with a subsequent growth of the endothelium of the sinus over it.



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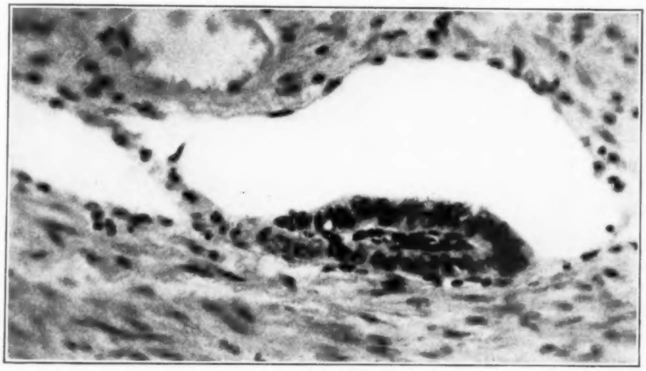

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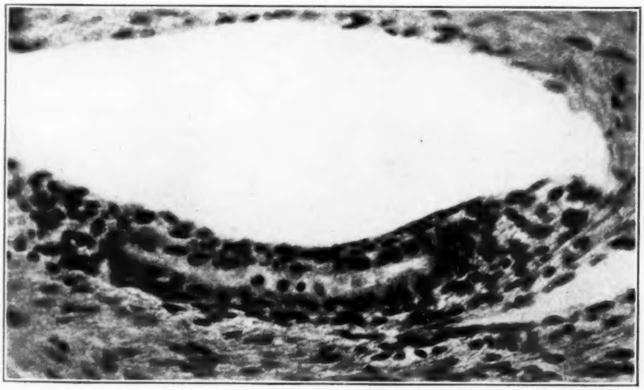

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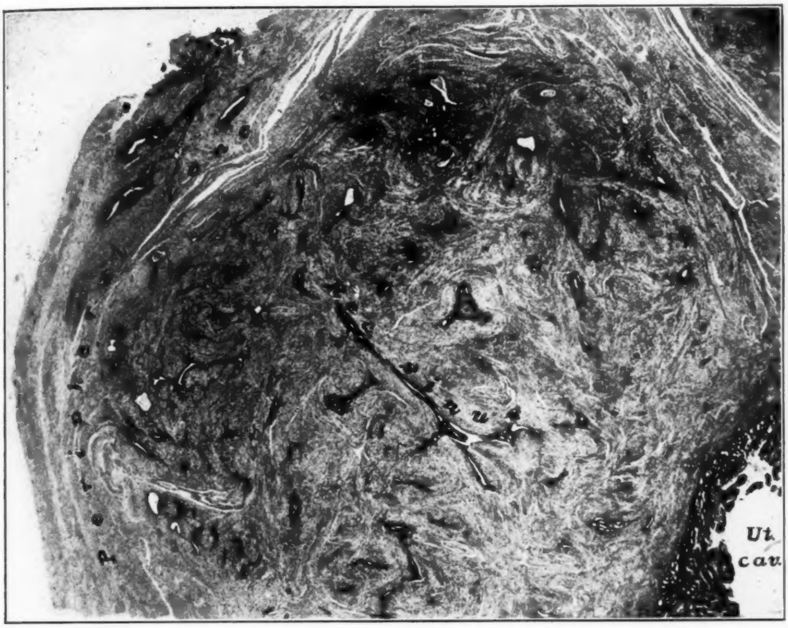
## Plate 31

Fig. 36. Photomicrograph ($\times$ 5) of a section of the left half of the posterior uterine wall (Case 4). An endometriosis is present, most marked in the radial zone. The distribution of the endometrial tissue in that zone is similar to the distribution of the bismuth in corresponding sections of uteri in which the veins have been injected. In this section many of the spaces occupied by the veins and venous sinuses of the uterine wall are filled with endometrial tissue (Fig. 38). An apparent receiving sinus (Fig. 39) is well outlined by this tissue. The entire uterus was cut into blocks and many sections were studied from each block and in only a small area was an invasion of the uterine wall by its mucosa found. I believe that the endometriosis of the radial zone shown in this photomicrograph possibly arose from this invasion. The endometriosis of the peripheral zone was more likely of embolic or metastatic origin. While the patient was menstruating at the time of the operation, only a very few of the areas of ectopic endometrial tissue showed a reaction to menstruation (Fig. 40).

Fig. 37. Photomicrograph ($\times$ 5) of a section of the right half of the posterior uterine wall at about the same level as that shown in Fig. 36. An endometriosis is not present in the radial zone of this section. An embolic implantation of endometrial tissue (End.) is situated in the arcuate veins (Figs. 49 and 50) and small emboli of endometrial tissue (E,E) are present in the veins of the peripheral zone (Fig. 51). There were two very interesting features of this uterus; one was the presence of an endometriosis of the direct type in the left half of the posterior uterine wall with only a very few evident embolic lesions and those in the peripheral zone; the other was multiple embolic lesions in the right half of the posterior uterine wall, most numerous in the peripheral zone with only a slight invasion of that half of the posterior uterine wall by the endometrial tissue from the opposite side.

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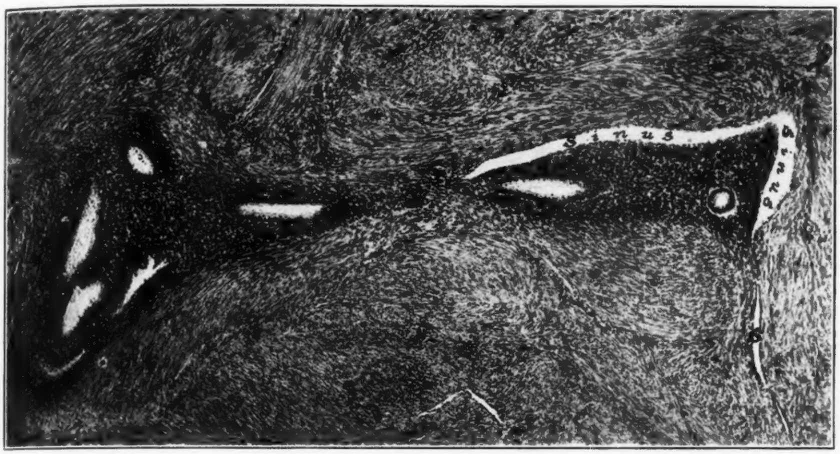
# PLATE 32

FIG. 38. Photomicrograph ( × 60) of a portion of the section shown in Fig. 36 illustrating a frequent type of lesion found in a direct or primary endometriosis. No trace of a vessel or sinus is evident in or about the endometrial area to the left. I believe that it fills the space originally occupied by a sinus and has either obliterated the lumen of the latter or pushed it to one side. The endometrial area to the right, a continuation of the former, projects into the lumen of a sinus like a sessile polyp but the surface of the latter is covered by endothelium. The endometrial tissue is invading the space occupied by the sinus in a retro-endothelial course, as has been so well described by Robert Meyer. I believe that this vessel is probably a venous sinus and not a lymphatic. It is conceivable that, should this endometrial tissue react to menstruation, blood containing bits of endometrial tissue would escape into the lumina of its tubules (see Fig. 40) and at times might rupture the overlying endothelium and escape into the lumen of the sinus.

FIG. 39. Photomicrograph ( × 25) of a portion of the "receiving sinus" shown in Fig. 36. The space occupied by the sinus is partially filled by endometrial tissue. The endometrial tissue has apparently invaded this sinus in a retro-endothelial course distorting the lumen (L.) of the latter as shown in Fig. 38 and in the manner of a direct or primary endometriosis. A direct invasion of this portion of the uterine wall by its mucosa was found and this is possibly a continuation of it. On the other hand, its histologic structure suggests a "canalized" endometrial thrombus which might have arisen from the implantation and growth of an embolus of endometrial tissue on the lining of the sinus with subsequent covering by endothelium. As will be shown, it is possible for the endothelium of a vessel or sinus to grow over endometrial tissue implanted in that vessel and give rise to lesions somewhat similar to those shown in this and the preceding illustration (see Figs. 41, 42 and 50).

FIG. 40. Photomicrograph ( × 60) of a section of a menstruating area in the endometriosis shown in Fig. 36. An endometrial cavity (dilated tubule) is filled with blood and bits of endometrial tissue. This blood might extend through the lumen of the tubule to other parts of the uterine wall invaded by this tissue. The endothelial lining of a venous sinus in this area or adjacent to it might be ruptured by the menstrual reaction, and menstrual blood carrying with it bits of the uterine mucosa, might escape into the venous circulation of the uterus. I have not been able definitely to demonstrate this in the menstrual reaction of endometrial tissue of a direct endometriosis but am confident that it does take place. For anatomic and physiologic reasons it should occur and I have observed it in an endometriosis of the posterior vaginal wall (Figs. 60 and 61).

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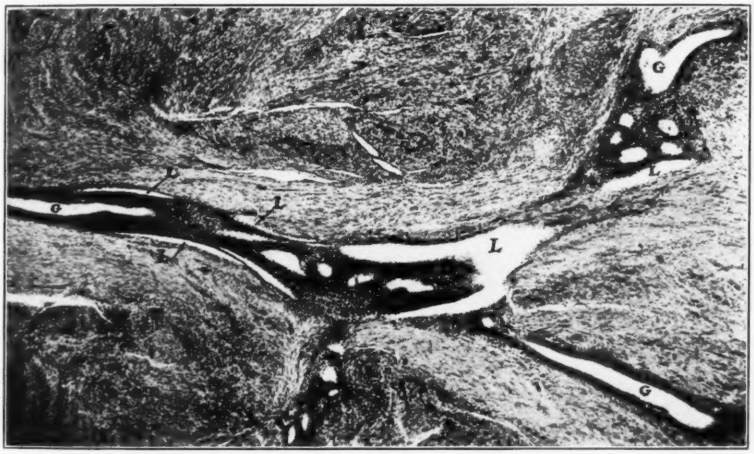


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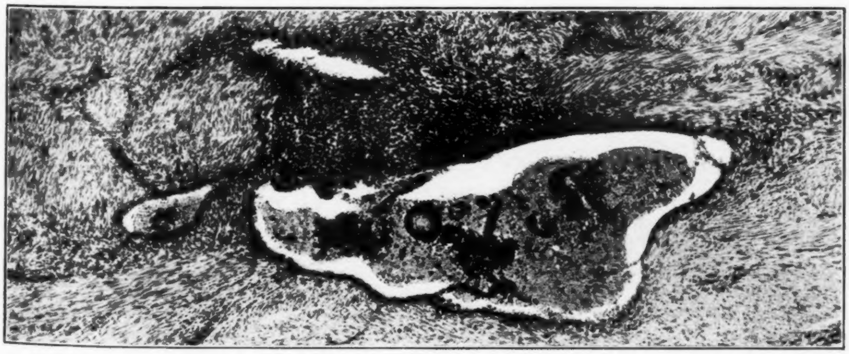

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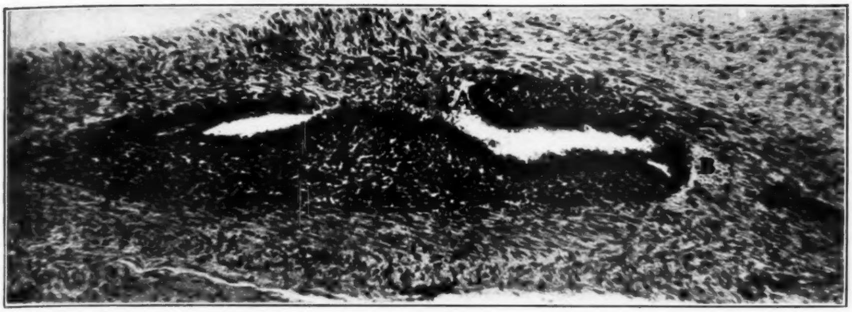
PLATE 33

FIG. 41. Photomicrograph ($\times$ 130) of an oblique section of a sinus almost completely filled with endometrial tissue (from the peripheral zone of the left half of the posterior uterine wall near the midline). Sufficiently complete serial sections were made to demonstrate that this tissue was not continuous with that in the radial zone or any outside of the sinus, but arose either from an implantation of a bit of endometrial tissue on the lining of this sinus or else from a metaplasia of its endothelial lining. I believe the former (see Figs. 42 and 43). In this section the implant is not attached to the wall of the sinus at A and B. The endometrial cavity, lined by epithelium, communicates with the lumen of the sinus at A. Should menstrual blood escape into this endometrial cavity, as shown in Fig. 40, it might carry with it bits of endometrial tissue into the lumen of the sinus and thence into the venous circulation of the uterus. At B the implant bulges into the lumen of the sinus but its surface is covered by endothelium, growing over it from that lining the sinus. This implantation lesion differs from that arising from the invasion of a sinus by endometrial tissue from without in that it is primarily intravascular while the latter is extravascular (retro-endothelial). Should complete endotheliazation of an implant occur, it might be histologically indistinguishable from that of a direct invasion and in its subsequent growth it might invade the lumen of the sinus as a retro-endothelial course and thus give rise to lesions similar to those shown in Figs. 36, 38 and 39. I have not been able to prove that this occurs.

FIG. 42. Photomicrograph ($\times$ 130) of the same sinus shown in Fig. 41 but at one end of the implant. At the right, the endometrial tissue is grafted on the wall of the sinus. To the left it is attached to the wall of the sinus by fibrin and endothelium apparently has begun to cover it. While the lesion shown here probably is an extension of the endometrial tissue shown in Fig. 41 it could well represent a stage in the implantation of embolic endometrial tissue lodging in a sinus (see Fig. 43).

FIG. 43. Photomicrograph ($\times$ 130) of the same sinus shown in Figs. 41 and 42 but beyond the implant. The sinus is partially filled with blood containing free bits of endometrial tissue possibly cast off by menstruation from the implant or from endometrial tissue elsewhere in the uterus as indicated below. Should this tissue become attached to the wall of this or another sinus and live, the lesions shown in Figs. 41 and 42 might arise. I believe that the lesion shown in Fig. 41 arose in this manner — from embolic endometrial tissue either cast off from another implant, from the menstrual reaction of a direct endometriosis with rupture into a vein, or from the menstruating uterine mucosa disseminating bits of endometrial tissue into the venous circulation of the uterus.

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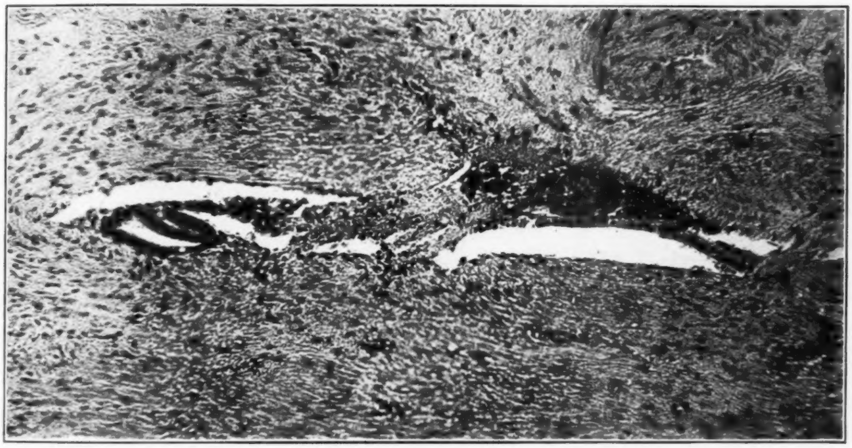

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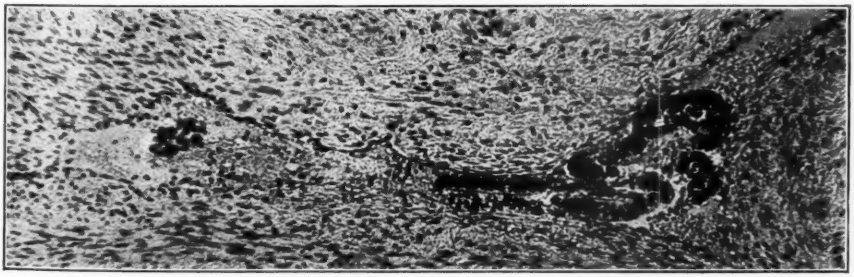

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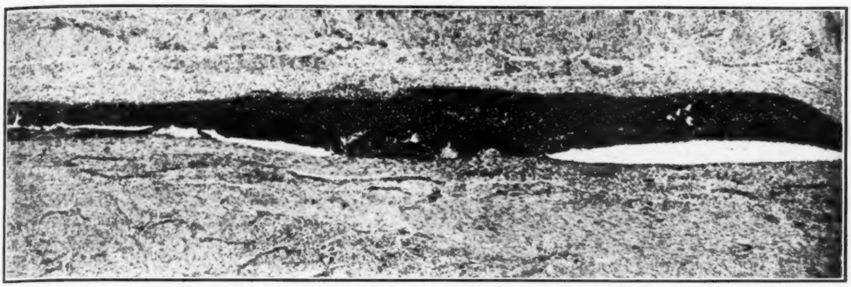
## PLATE 34

FIG. 44. Photomicrograph ($\times$ 25) of an arcuate vein in the lower portion of of the posterior uterine wall. The lumen of the vessel is almost completely filled with endometrial tissue. It resembles the lesion of a direct endometriosis but is also similar to that shown in Fig. 41. This was the first section obtained after trimming the block and therefore it was impossible to ascertain whether or not it is of embolic origin. My reaction is that it is of embolic origin. This lesion was followed through the block until it disappeared (see Figs. 45 and 46).

FIG. 45. Photomicrograph ($\times$ 25) of the same vein shown in Fig. 44 but deeper in the block. It differs from the latter in that the lumen of the endometrial tubule (cavity) communicates with that of the vein at A and also at B. The latter might be an artefact (torn in cutting) but not the former (see Fig. 47).

FIG. 46. Photomicrograph ($\times$ 25) of the vein or branches of the same shown in Fig. 44. A little beyond this level the lesion disappeared. The lesions are similar to those shown in Fig. 38 and circumstantial evidence indicates that the latter might have arisen from the direct invasion of the uterine wall by its mucosa.

FIG. 47. Photomicrograph ($\times$ 130) of the portion of the vein indicated by A of Fig. 45. The endothelial lining of the vessel is well shown in the upper portion of the right half of the photomicrograph. The lumen contains blood and is loosely filled with stroma. The endometrial cavity to the left empties into the lumen of the vein. It is not an artefact. It represents either the incomplete grafting of an endometrial implant as shown in Fig. 41 or else the endothelial covering of the endometrial tissue of a direct endometriosis has been destroyed by menstruation. My present reaction is that it is the former (compare with Fig. 41 which we know is of embolic origin).



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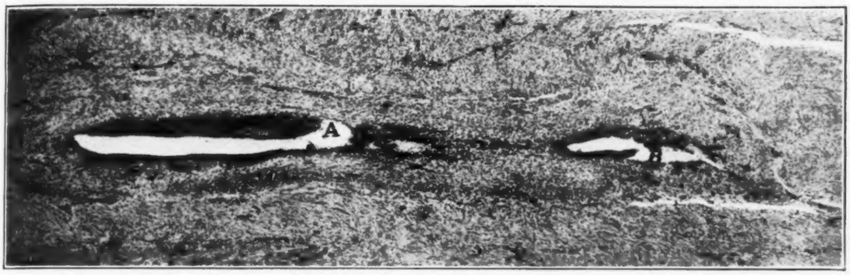


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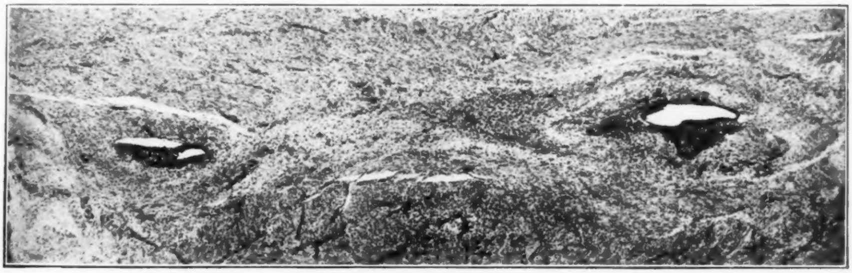

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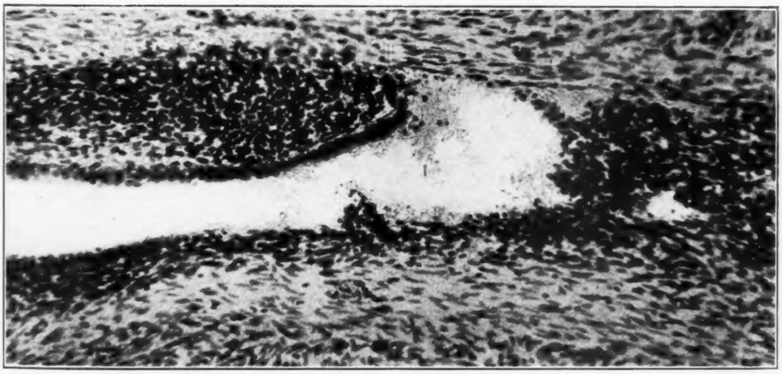

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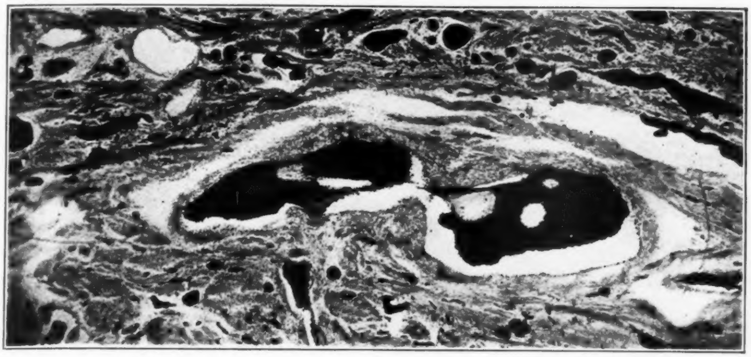
FIG. 48. Photomicrograph ($\times$ 25) of a section of the uterine wall showing an arcuate vein cut obliquely; veins injected with bismuth. The blood from the peripheral and radial zones of the uterus empties into the arcuate veins and is conveyed by these to the venous circulation outside of the uterus. Bits of endometrial tissue carried by menstrual blood into the ruptured venous sinuses of the uterine mucosa (Figs. 22 and 23) would reach these veins through the receiving sinuses (Figs. 9 and 10). These bits might become implanted in these veins (Fig. 49) or under the varying changes in the pressure of the venous circulation of the uterus they might be carried into vessels of the peripheral zone (Figs. 11, 51 and 65) or even escape into the venous circulation outside of the uterus.

FIG. 49. Photomicrograph ($\times$ 25) of a section of the arcuate vein shown in "End." of Fig. 37. It is almost an exact duplication of the vein shown in Fig. 48 except that the injection mass is replaced by endometrial tissue. Sufficiently complete serial sections were made of this block to demonstrate that the endometrial tissue in the vein did not arise from the invasion of the vessel by endometrial tissue outside of it. The endometrial tissue in this section is entirely intravascular and must have arisen either from a "metaplasia" of the endothelium of the vessel or else through the implantation and growth of an endometrial embolus similar to those found floating about in lumina of other vessels (see Figs. 43, 53 and 65). The endometrial tissue of this implant has the same structure as that of the mucosa lining the uterine cavity (Fig. 55) and that found in the endometriosis of the opposite side of the posterior uterine wall shown in Figs. 36, 38 and 39.

FIG. 50. Photomicrograph ($\times$ 25) of another section of the arcuate vein shown in Fig. 49. The lesion differs from the preceding one in a very important feature. The endometrial tissue in the former is entirely intravascular while that in this section is partially retro-endothelial due to the growth of the endothelium of the vessel over the end of the endometrial tissue projecting into the lumen of the vein (to the left) just as endothelium covers a mural thrombus. Should the endothelium grow more rapidly than the endometrial tissue, it might cover the entire unattached portion of the endometrial implant. Should bits of endometrial tissue be cast off by the menstrual reaction of such an implant they would escape into the venous circulation of the uterus. Some of the endometrial emboli in this specimen might have had such an origin (Fig. 65).

FIG. 51. Photomicrograph ($\times$ 25) of a section of the peripheral zone of the uterine wall from the same block shown in Fig. 37 and very near the latter. Bits of endometrial tissue (End.) are present in the veins of this section, some lying free in the lumen of the vessel and others attached to its wall. (For a higher magnification of similar lesions in another section of the peripheral zone see Fig. 65.) These bits of endometrial tissue must have arisen from the dissemination of similar tissue into veins of the peripheral zone from the menstrual reaction of an endometrial implant such as that shown in Figs. 41 and 49, or from the menstrual reaction of the uterine mucosa discharging menstrual blood into a ruptured mucosal sinus as has been demonstrated, or from a similar reaction of the endometrial tissue of a direct endometriosis which must occur but which I have not been able definitely to prove.



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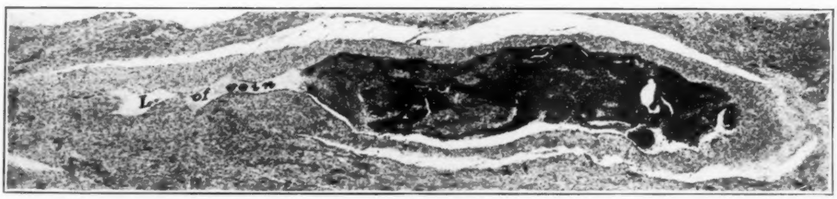


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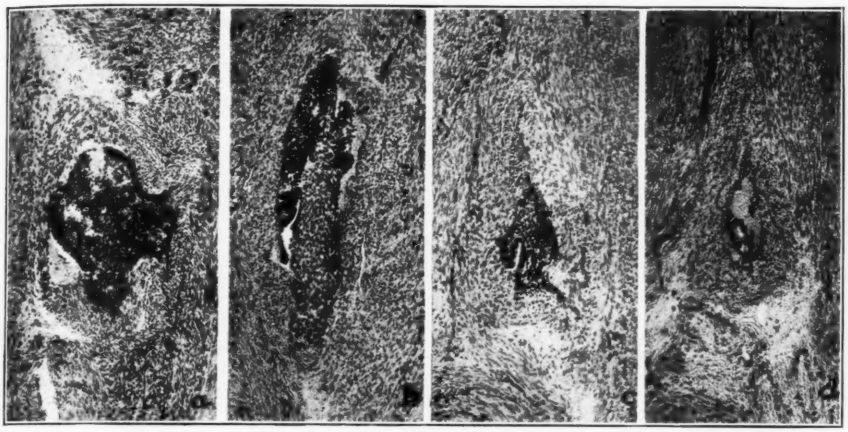
## PLATE 36

FIG. 52. Four photomicrographs ($\times$ 60) from a series of sections showing the appearance, at different levels, of an embolic implantation of endometrial tissue in a vein or sinus of the peripheral zone of the right half of the posterior uterine wall. *a* shows the condition found near one end of the "endometrial plug" and *d* that found near the other end. In *a* and *d* there is no evidence of endotheliazation while in *b* and *c* the implant is apparently partially covered by endothelium. Many lesions similar to this one were found in the peripheral zone of the right half of the posterior uterine wall.

FIG. 53. Three photomicrographs of sections of veins in the right half of the posterior uterine wall. *a* ($\times$ 60) shows a bit of endometrial tissue surrounded by blood in the lumen of a large vein near the lateral surface of the uterus, very close to the uterine plexus and therefore almost in the venous circulation outside of the uterus by which it could easily be carried to the lungs. *b* ($\times$ 130) shows a bit of uterine epithelium attached to or resting on the lining of a sinus of the radial zone. It is situated between the mucosa lining the uterine cavity and the arcuate veins and is in a channel by which endometrial tissue escaping from the uterine mucosa would reach these veins. *c* ($\times$ 60) shows an embolic endometrial implant in a sinus of the peripheral zone with no evidence of endotheliazation.

FIG. 54. Photomicrograph ($\times$ 25) of an embolic growth of endometrial tissue in a receiving sinus of the right half of the posterior uterine wall. It more than suggests that this endometrial tissue primarily escaped into this sinus from a sinus of the uterine mucosa and that similar bits might have reached the arcuate veins into which the receiving sinuses empty and from the arcuate veins escaped into the peripheral veins of the uterine wall. I believe that the embolic lesions of endometrial tissue in the veins and sinuses of the right half of the posterior uterine wall primarily arose from the menstrual dissemination of bits of the uterine mucosa into the venous circulation of the uterus rather than from a similar reaction of the endometrial tissue of a possible direct endometriosis.



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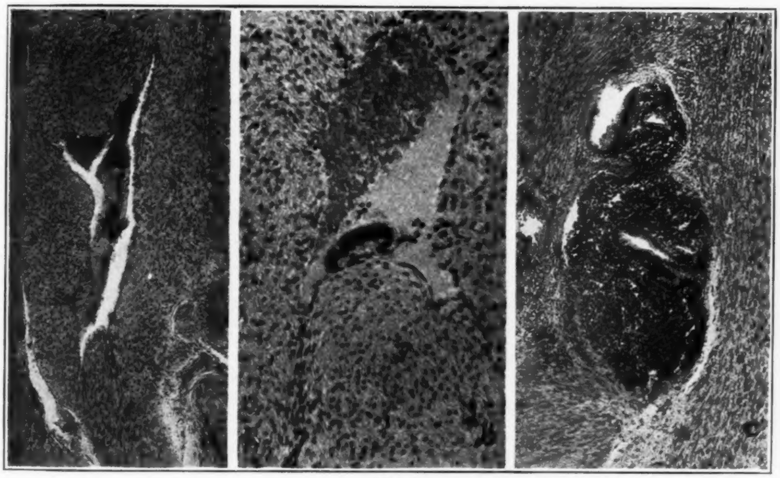

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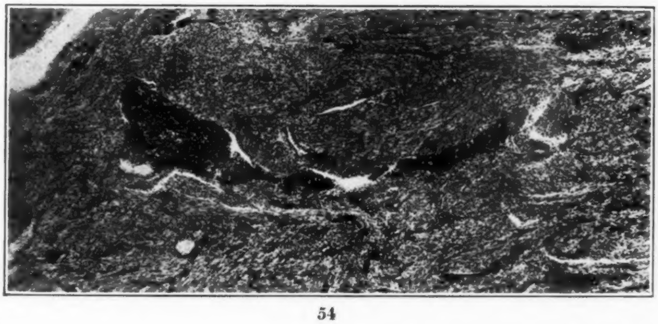

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PLATE 37

FIG. 55. Photomicrograph ($\times$ 25) of the menstruating mucosa lining the uterine cavity (Case 4). A mucosal sinus is present containing blood and a bit of endometrial tissue (End.). It might be argued that the latter is an artefact in this instance and gained access to the lumen of the sinus from the trauma of the operation or in cutting the blocks from the hardened specimen. Should such a bit escape into the lumen of a mucosal sinus during menstruation, as they do, we might expect to find similar fragments in the sinuses of the uterine wall including the receiving sinuses and also in the arcuate veins and even those of the peripheral zone. As has been shown, not only were these found but also the actual implantation and growth of this tissue in these vessels, thus demonstrating that bits of endometrial tissue disseminated by menstruation are sometimes alive and capable of becoming implanted on the endothelial lining of the veins and venous sinuses of the uterine wall.

FIG. 56. Photomicrograph ($\times$ 10) of a section of a portion of the anterior uterine wall near one of the cornua with the anterior layer of the broad ligament fused to it by the endometriosis in this situation. The anterior cul-de-sac was partially obliterated by a peritoneal endometriosis fusing the anterior surface of the uterus with the anterior layer of the broad ligament and the peritoneum covering the bladder. The lesion was most marked about the uterine cornua. The lumen of the cul-de-sac is patent at both ends of the section (*a* — *a* and *b* — *b*) but is occluded in the center. It would seem that the lesion started at X possibly on or near the peritoneal surface of the uterus and radiated in all directions invading the uterine wall and the anterior layer of the broad ligament to about the same depth. The entire uterus was cut into blocks and many sections were studied from each block. No endometrial tissue was found in the anterior uterine wall except similar to that indicated in this section. Definite embolic lesions, like those in the right half of the posterior uterine wall, were not found in any portion of the lesion. The endometrial lesions in this section and in all others from this portion of the specimen showed an endometriosis of the invasive type apparently spreading from or near the peritoneal surface of the uterus. In a few areas a possible communication of the lumen of an endometrial tubule with that of a vessel was suggested, thus indicating a possible metastatic origin. Three theories may be considered for the origin of the endometrial tissue in this situation. 1. From the stimulation of potential endometrial tissue in the serosa. 2. From the implantation of endometrial tissue on the peritoneum from menstrual blood escaping into the cul-de-sac. Both tubes were patent and endometrial tissue was not found in the ovaries. 3. From endometrial emboli lodging in subperitoneal vessels of the peripheral zone of the anterior uterine wall. I have been unable to determine its origin but believe that primarily it must have been either an implantation or an embolus.

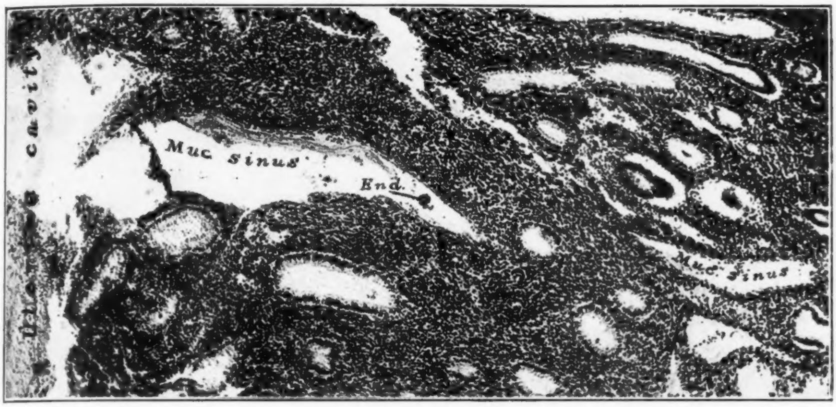


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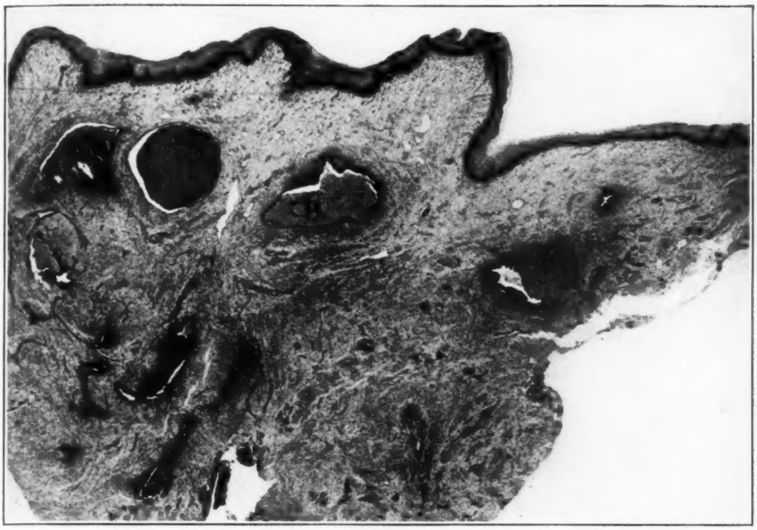
PLATE 38

FIG. 57. Photomicrograph ($\times$ 10) of a longitudinal section of a portion of the posterior vaginal wall near the cervix (Case 4). An endometriosis is present showing a variety of endometrial lesions including cavities filled with blood (patient menstruating at the time of the operation). The endometrial tissue in this situation may have been primarily derived from a direct extension downward from that in the cul-de-sac or emboli in the venous circulation of the uterus may have been carried to the vaginal wall through the vaginal veins. I believe that the former is the more likely source.

FIG. 58. Photomicrograph ($\times$ 25) of a portion of the section shown in Fig. 57. A cavity is present partially lined by epithelium and filled with blood containing bits of endometrial tissue cast off by the menstrual reaction. Bits of endometrial tissue, End., identical with those in the cavity are present in small veins to the right of this cavity. Some of these fragments lie free in the blood of these veins and others are attached to or implanted on the walls of the vessels (Figs. 59 and 65). What is the origin of the endometrial emboli in these vessels? Are they metastatic from the uterus or were they disseminated from the ectopic endometrial cavities in the vaginal wall? I believe the latter (see Figs. 60 and 61).

FIG. 59. Two photomicrographs ($\times$ 130) of portions of the section shown in the preceding illustration. *a* is a longitudinal section of a vein with a small bit of endometrial tissue lying free in its blood, *b* a cross-section of a vein with a strip of epithelium "buckled" in its lumen and *c* a cross-section of a vein distended by endometrial tissue growing in the vessel. For a photomicrograph of another vein containing endometrial tissue see Fig. 66.



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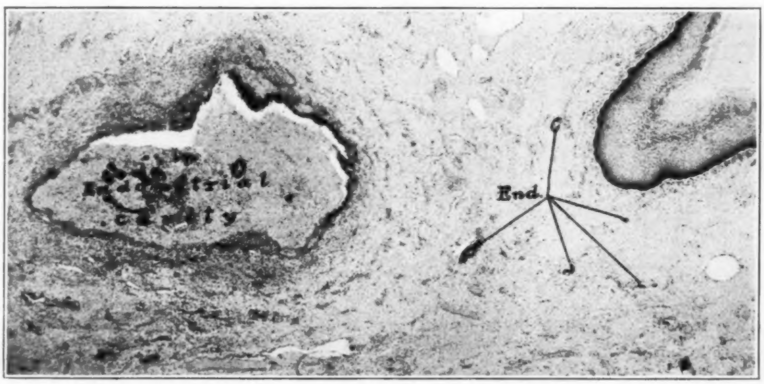


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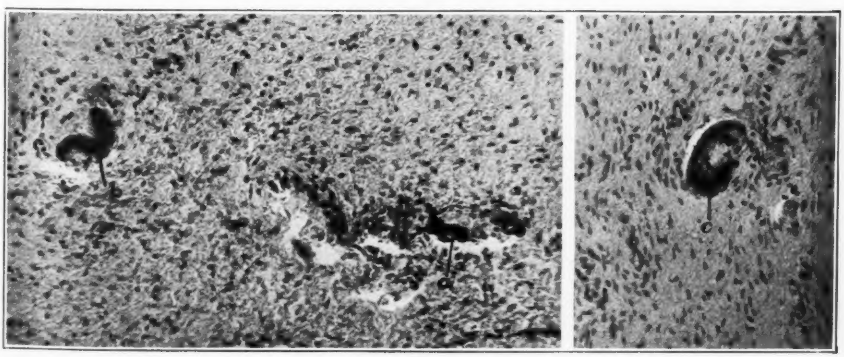

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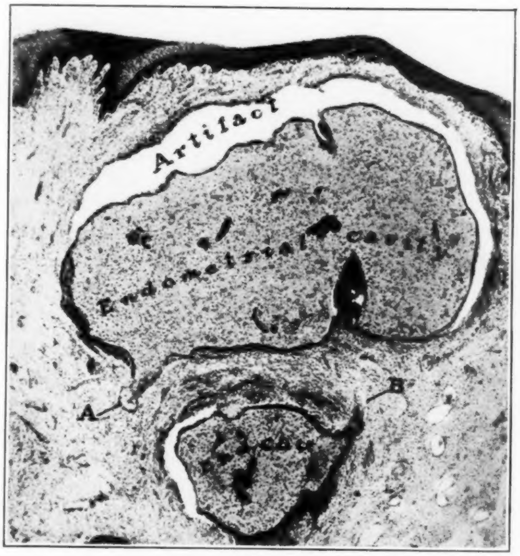
## Plate 39

Fig. 60. Photomicrograph ( × 25) of a section from the same block as the preceding. Two endometrial cavities are present, the larger just beneath the vaginal mucosa. Both of these cavities are filled with blood (menstrual) containing cast-off bits of endometrial tissue. The smaller cavity has ruptured into a vein at B and some of its contents are escaping into the lumen of the vein. The larger cavity has also ruptured into a vein at A and some of its contents are likewise escaping into the lumen of this vein (see Fig. 61).

Fig. 61. Photomicrograph ( × 310) of a portion of the section shown in A of Fig. 60. The endometrial cavity, above, has ruptured through the endothelial lining of the vein and some of the contents of the former have escaped into the lumen of the vein. I believe that the endometrial emboli in the veins of the vaginal wall already shown (Figs. 58 and 59) had a similar origin. As some of these emboli have become implanted on the lining of these vessels (see Figs. 59 and 66) it is again evident that bits of endometrial tissue, cast off by menstruation, are sometimes alive and capable of becoming implanted on the endothelial surface of veins. If bits of endometrial tissue escape into veins during the menstrual reaction of ectopic endometrial tissue in the vaginal wall, we would expect that a similar condition might arise in ectopic endometrial tissue in any situation including a direct or primary endometriosis of the uterus.

Fig. 62. Photomicrograph ( × 130) of a cross-section of a vein in the vaginal wall at a distance from the endometriosis shown in the preceding illustrations. The vein was situated in the lower part of the portion of the vagina which had been excised. A mural thrombus is present containing a bit of "endometrial tissue." We would expect that similar bits of endometrial tissue might escape into the general venous circulation and be carried to the lungs. The patient had as a postoperative complication, "a bronchopneumonia" from which she recovered. Is it possible that this might have been due to a "shower of endometrial tissue" disseminated by the trauma of the operation?

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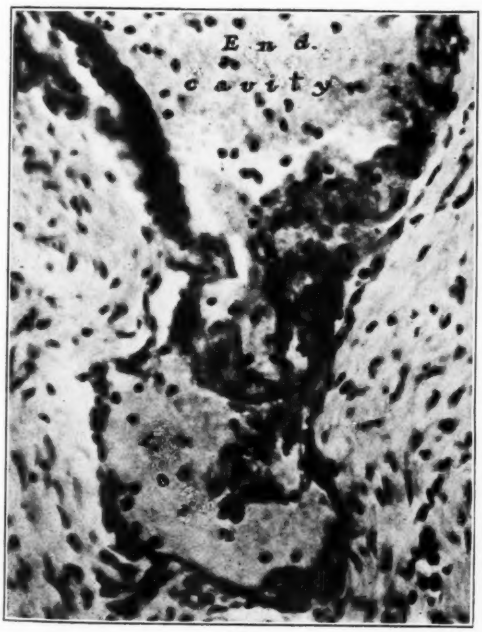


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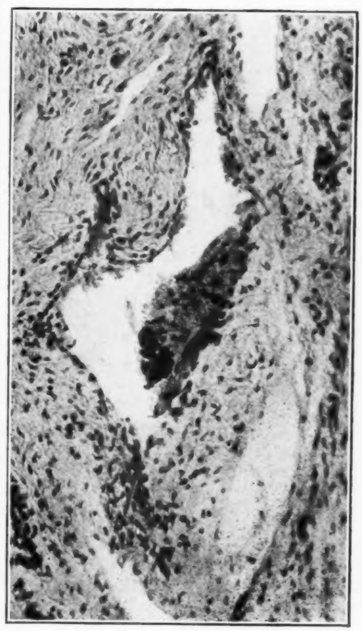

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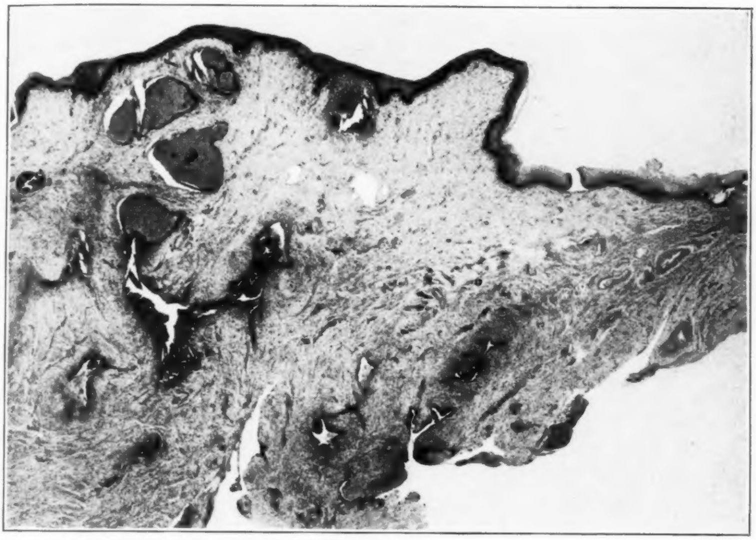
PLATE 40

FIG. 63. Photomicrograph ( $\times$ 10) of another section of the vaginal wall again showing many endometrial lesions, including one just beneath and eroding the vaginal epithelium.

FIG. 64. Photomicrograph ( $\times$ 25) of the vaginal wall from the same block as the preceding illustration demonstrating the erosive action of endometrial tissue on the vaginal epithelium. The endometrial cavity contains blood and bits of endometrial tissue set free by the menstrual reaction. In time the overlying vaginal epithelium might rupture and the contents of the cavity would escape into the vagina just as similar cavities in the ovary or any pelvic structure might rupture and their contents escape into the peritoneal cavity. The hemorrhagic areas of the posterior vaginal wall (see Fig. 67) are due to the accumulation of menstrual blood in these subepithelial endometrial cavities.



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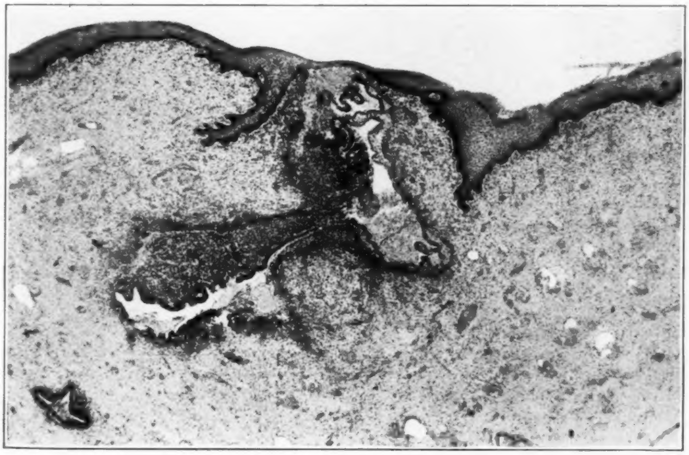

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## Plate 41

Fig. 65. Colored photomicrograph ( $\times$ 130) of a portion of the peripheral zone of the right half of the posterior uterine wall (Case 4). The patient was operated upon the second day of menstruation. Fragments of endometrial tissue (emboli) are present in two veins (probably two sections of one vein). It is natural to assume that they were set free by the menstrual reaction of either an embolic growth in a vein or else from the mucosa lining the uterine cavity. The implantation of such emboli in veins would give rise to lesions similar to those shown in Figs. 41, 49 and 52.

Fig. 66. Colored photomicrograph ( $\times$ 130) of a portion of the posterior vaginal wall (Case 4) showing endometrial epithelium implanted in a vein, one of the veins indicated in Fig. 58 (see also Figs. 59, 60 and 61).

Fig. 67. Cervix and portion of the posterior vaginal wall (natural size) showing the characteristic appearance of the endometriosis in this situation. The hemorrhagic elevations are due to the accumulation of menstrual blood in subepithelial endometrial cavities (see Figs. 63 and 64).

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# THE FINER VASCULAR CHANNELS OF THE SPLEEN *

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## Introduction

Among those organs and tissues of the body of which the essential anatomy and physiology, in both health and disease, still remains more or less obscure, the spleen is conspicuous as an organ of large size about whose finer structure considerable uncertainty still remains. Keen students of the structure of the spleen have not been lacking. As one reads the contributions of Gray,[1] of Mall[2] and, above all, of Weidenreich,[3] he gains the impression that the subject is already quite fully elucidated. Unfortunately the subsequent publications of Helly,[4] of Mollier[5] and the recent contribution of Thoma[6] have called into question Weidenreich's description of the finest vascular channels of the spleen. The anatomy of the circulation in this organ is of fundamental importance to all other considerations which may concern its physiology, its pathologic anatomy or its abnormal function. Weidenreich found that most of the arterial capillaries of the spleen open out into spaces of the spleen pulp so that the blood circulation is an open one. Helly and, more recently, Thoma oppose this view absolutely and conclude that the arterial capillaries open directly into the venous sinuses and that the blood circulation is a closed system similar in this respect to the blood circulation of other organs and tissues. The still more recent paper of Robinson[7] lends support to the advocates of the open circulation.

A brief study of the ordinary sections of spleen as they have usually been prepared for histologic study will quickly show that the finer structure of this organ cannot be recognized in such specimens. One can distinguish follicles and pulp, arterioles near the center of the follicles and occasionally in the pulp he may distinguish a venous sinus filled with blood and outlined by pulp cords in the substance

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of which there is also blood. Vascular channels connecting the arterioles with the venous sinuses are left to the imagination of the student of such a specimen. Yet it must be evident that along these finest channels the most important activities of the spleen are accomplished.

## Technic

The human spleens used in this study have been obtained in part as fresh surgical material from patients subjected to splenectomy for various reasons and in part from early necropsies. The surgical specimens usually presented distinct abnormalities but offered the advantage of excellent fixation in the freshest possible state. The spleens from necropsy were removed from one to several hours after death, but in many instances presented no recognizable pathologic changes. In either case the procedure followed in the laboratory has been essentially the same. After rapid gross description, including weight and measurements, consistence and external appearance, one sears the capsule at one end and, by puncturing with a sterile glass capillary pipette, removes a sample of pulp for aerobic and anaerobic bacteriologic culture. One next cuts off a portion of the organ at this end, placing thin slices in various fixing fluids for histologic study in the undistended state and also preparing smear preparations of the pulp which may be variously stained in the search for special types of cells or parasites. Next, by blunt dissection of the fat at the hilum, one isolates an arterial branch which enters near the intact end of the organ and ties into this vessel a suitable glass cannula. Through this are injected at moderate pressure perfusion fluids, first Locke's gelatin solution (sodium chloride 9.2 gm., sodium bicarbonate 0.05 gm., potassium chloride 0.1 gm., calcium chloride 0.15 gm., gelatine 2.5 gm., water 1000 cc.), then a small amount of physiologic salt solution and finally Helly's fixing solution (potassium bichromate 2.5 gm., sodium sulphate 1 gm., mercuric chloride 5 gm., water 100 cc., formalin 5 cc.) until at least a small portion of the spleen substance shows the yellow discoloration due to this fluid. The artery is then clamped and the cannula removed. Next one inserts a larger cannula into one of the venous branches coming from near the middle of the organ and carries out a venous perfusion in a similar manner. It is sometimes well to ligate lightly the severed end of the spleen to prevent the too rapid loss of fluid in the venous

perfusion. A considerable portion of the organ should remain visibly distended. After ligating the vessels the entire mass is immersed in Helly's solution for four to six hours, then divided into slices, the portion perfused through the artery being placed in one bottle, the vein-perfusion slices in another and portions of the remaining substance in a third. One is thus provided with a variety of material from the same spleen for study in sections. The subsequent handling presents nothing out of the ordinary except that the mercury must be removed and the serial paraffin sections must be cut at a thickness of 4 microns or preferably 3 microns. A firm paraffin should be used so that portions are not displaced by the knife in cutting. Excellent technic is essential here.

The study of human material has been supplemented by the study of animal spleens from rabbits, guinea-pigs and dogs, most of the material being from rabbits. Here it has been possible to use not only normal organs but also to utilize the spleens of animals in which distinct pathologic alterations in the spleen have been experimentally induced; furthermore, to employ intravital injection of foreign substances such as nucleated erythrocytes from birds and suspensions of opsonized bacteria as well as to perfuse the spleen with the fixing fluids during the life of the animal. Such injections of the living spleen are best done by inserting a very fine glass cannula into one of the short gastric branches of the splenic artery and injecting the fluid through this back into the main artery from which it passes to the spleen by the force of the blood pressure of the animal.

The cannula is made from ordinary soft glass tubing by drawing out one end to a very fine capillary and fusing on a short side-arm near this tip. The capillary tip has to be smoothed in the flame with great care so that the edges of the minute tip will not too readily cut the vessel wall. This smoothing is accomplished by heating the tip in the gas flame while blowing a current of air through the lumen to prevent sealing of the tip. When properly smoothed, the glass tip shows rounded free edges when examined under the low power of the microscope. Its roughness may also be tested by scratching the surface of the finger nail. The lumen at the tip should not be narrowed. Some practice may be required before one is successful in making serviceable cannulas.

## The Arterial Capillaries in Serial Sections

The arterial capillaries of the spleen may be classed for purpose of description into three groups: (1) follicular capillaries, (2) capillaries of marginal zone and (3) capillaries of the pulp cords.

The follicular capillaries come off in considerable numbers from the follicular artery and pass almost straight through the substance of the follicle to its periphery, where they pass only a short distance beyond the capsule. Some of these vessels evidently anastomose by means of short communicating branches at the level of the capsule. The terminal twigs extend into the less compact marginal zone where they terminate by numerous small openings into the spaces of the spleen pulp of this marginal zone, always at considerable distances from the nearest venous sinuses. The endothelial cells lining the terminal portion are branched and their processes are in continuity with similar processes of the pulp cells proper. Evidently the state of the terminal endothelium is subject to variation. Sometimes the openings into the pulp appear large and permit ready escape of corpuscles. At other times the openings appear to be constricted by the contraction of the endothelial cells so that erythrocytes are held back and even the passage of liquid is impeded. In such cases the terminal portion may be distended in the form of an olive or an acorn to present the picture of an ampulla. These capillaries evidently provide a rich nourishment for the follicles. The plasma passes through the capillary wall as lymph to bathe the reticulum of the follicle and the closely packed proliferating cells in the meshes of this reticulum. It is probable that dissolved substances, including toxic agents, escape with this fluid and come into direct relation with the lymphocytes and reticular cells of the follicle. Relatively few blood cells escape from the vessels here. On this account, the terminations of these vessels at the marginal zone contain blood excessively rich in formed elements and poor in plasma. These formed elements have to escape through minute openings between the branches of endothelial cells into the pulp spaces. They thus come into very intimate contact with the terminal capillary endothelium and with the immediately continuous reticulo-endothelial cells of the pulp, which is loosely arranged with wide meshes in the rather broad marginal zone about the follicle. Abnormal blood cells, especially those which have become somewhat adhesive through injury, are readily phagocytized

by the endothelial and pulp cells at this place. Those blood cells which pass this searching inspection escape from the pulp through the abundant stomas of Mollier by which the pulp spaces freely communicate with the lumina of the venous sinuses.

The capillaries of the marginal zone are given off from arterial branches which run out from the follicle into the pulp. Such a branch gives off from three to ten divisions which quickly terminate in the substance of the marginal zone about the follicle and one or two much longer terminal capillaries which extend peripherally into a pulp cord between venous sinuses. The centripetal twigs are usually much curved and elusive in serial sections. They are rather short and do not anastomose. They often branch dichotomously just before terminating and the very tip is usually distended to a thin-walled ampulla from which many narrow and irregular clefts lead into the pulp spaces of the marginal zone. The axis of the terminal ampulla is nearly tangential to the capsule of the follicle. These vessels are somewhat larger and more readily distended than are the follicular capillaries. Except for the terminal portion their walls are conspicuously thick, consisting of a layer of rather large endothelial cells with nuclei standing out in the lumen. This endothelium is surrounded by a syncytium of rather large and compactly arranged cells which also appear to be endothelial in type but with minute lymph spaces between them. These may be in several layers and by their more compact arrangement are conspicuous in the less compact pulp of the marginal zone and pulp cords. They constitute the arterial sheathes of Schweigger-Seidel.[8]

The capillaries of the pulp cords are derived from the same trunk as the preceding group. They are more easily followed in serial sections of the distended spleen, because they often run fairly straight or curve chiefly in one plane so that three to five sections may sometimes suffice to trace the course of such a capillary from its origin by final branching of its trunk vessel to its terminal extremity in the pulp between the venous sinuses at a distance from the follicles. In the distended spleen such a vessel is wide enough to contain a single row of erythrocytes. Its termination presents some variations. In some instances there is an olive-shaped terminal ampulla twice as wide as the capillary proper and outlined by strands of endothelial cells between which numerous clefts permit communication with the adjacent pulp spaces. In other instances the vessel opens directly

into the pulp without evident ampulla. Occasionally, also, one can trace such a pulp capillary as a definite vessel quite to the wall of a venous sinus into which it opens by an extremely narrow cleft between endothelial cells. This type of termination, first clearly recognized by Weidenreich, appears to be the exception rather than the rule, even in the termination of the pulp cord capillaries. The size of the opening is small and ordinarily of the same order as the stomas in the wall of a venous sinus; it is by no means similar in structure or in functional capacity to the capillary-vein union in other organs. These pulp-cord capillaries are formed of a single layer of endothelial cells with nuclei projecting into the lumen. External to this wall is a sheath of spleen pulp similar to that of all the pulp cords, consisting of branched reticular cells enclosing pulp spaces which lead over to venous sinuses on either side. Evidently much of the plasma escapes from the lumen of the pulp-cord capillary in its course so that concentrated corpuscles come to the termination of it. Capillaries of this group although rather conspicuous and more easily traced than those of the first two groups, are much less numerous and evidently far less significant for the function of the spleen.

The above anatomic description, based upon the study of serial sections of distended human and rabbit spleens, differs little from the description of Weidenreich. The method is open to certain legitimate criticisms which have been previously voiced in regard to studies of this kind. First, distension of the spleen by perfusion through the vessels may rupture the more delicate structures and produce artificial openings and passages where naturally none exists. This criticism has been urged against all conclusions based upon anatomic injections of the spleen. The point of this criticism is largely lost, however, when the perfusion is carried out by introducing the perfusion fluid through a branch of the splenic artery in the living animal and permitting it to be forced into the spleen at the arterial pressure. A second criticism appears at first somewhat more serious, namely, that in very thin serial sections of the distended spleen the individual elements, cells, nuclei and fibers are irregularly displaced by the knife of the microtome, so that one is not able to recognize the successive portions of the same structure in successive sections. In our series of approximately a hundred spleens there are excellent illustrations of this difficulty. Only when the perfusion fixation has succeeded well and the dehydration and embedding and the micro-

tome technic have been quite perfect can one expect to obtain useful serial sections of spleen at a thickness of 3 microns. We do feel, however, that this difficulty has been surmounted in the technical handling of some of our material and we regard the illustrations submitted herewith as justification for this opinion.

## Experimental Demonstration of the Open Capillary Circulation of the Spleen

Weidenreich appears to have sensed the weakness of unsupported morphologic evidence and he therefore undertook to furnish experimental evidence as well. He injected the blood of a bird into the ear vein of a rabbit, waited several minutes, killed the rabbit and prepared sections of the spleen. He found the nucleated erythrocytes of the bird in the pulp spaces and concluded that they had reached this situation from the open ends of arterial capillaries. Helly who repeated these experiments reached a quite contrary conclusion. He believed that the bird's erythrocytes passed from the arterial capillaries into the venous sinuses and from here through the clefts in the sinus walls into the pulp. The demonstration by Mollier of the abundant stomas in the sinus walls has been accepted by some modern authors as strong support for Helly's interpretation. There can be no doubt that bird's erythrocytes can be found in such preparations actually half way through the wall of a venous sinus, caught in transit by the fixation, passing, according to Weidenreich, from the pulp spaces to the sinus lumen, or, according to Helly, from the sinus lumen to the pulp space. It seemed to us that this question might be elucidated by fixing the spleen at a very much shorter interval after introducing the foreign corpuscles. Therefore, instead of injecting them into the ear vein, we introduced the foreign corpuscles into the splenic artery through one of its short gastric branches in the living animal and then followed this injection by perfusion with salt solution and fixing fluid (Helly's formalized Zenker solution) within one minute. We hoped by such technic to be able to find the bulk of the nucleated erythrocytes either in the sinuses with few or none in the pulp if Helly's view should be correct, or on the other hand, if Weidenreich were right, to find them abundant in the pulp and few in the sinuses. After a few trials to perfect the technic it was possible to obtain quite convincing preparations

showing abundant bird's corpuscles caught in the pulp meshes, especially in the marginal zones, a few bird's corpuscles passing through the stomas in the sinus walls and very few such corpuscles within the sinus lumen (Fig. 16). Evidently the corpuscles in the stomas are in transit *from the pulp spaces to the sinus lumen.*[9]

The results of these experiments confirm in a satisfactory manner the interpretation which has been reached by the study of the serial sections.

## Summary

1. There is proposed a technic for the fixation and preservation of human spleens obtained by surgical splenectomy or at necropsy, which makes possible a more illuminating study of the structure of this organ.

2. For the study of animal spleens a modified perfusion technic is described, in which the perfusion fluid or experimental injection material is introduced into a short gastric branch of the splenic artery.

3. The blood circulation in the spleen is essentially an open circulation in that the pulp spaces constitute the connecting channels through which the blood passes from arterial capillary to venous sinus, a mechanism which brings about intimate contact of the blood plasma with the cellular elements of the follicle and a similar intimate contact between the formed elements in the blood and the reticulo-endothelial cells of the marginal zone and the pulp cord.

4. It is believed that an adequate understanding of the splenic circulation and the application to normal and pathologic material of the methods employed here may be of service in advancing our knowledge concerning the normal physiology, the pathologic anatomy and the abnormal function of this rather enigmatic organ.

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## DESCRIPTION OF PLATES

### Plate 42

Fig. 1 (Photomicrograph). — Capillary vessels of the splenic follicle of the rabbit. The living spleen was perfused with Locke's solution followed by Helly's fluid to fix it in the distended state. The excentric follicular artery remains widely dilated and several thin capillaries are visible radiating from near the center of the follicle. Some of the latter show a distinct lumen. (Hematoxylin-eosin stain.)

Fig. 2 (Photomicrograph). — Termination of a follicular capillary at margin of follicle in human spleen. The patient was a child of eight years presenting the manifestations of purpura with a marked platelet deficiency, for which splenectomy was performed. This section is from the portion distended by arterial perfusion stained with phloxine-azure B. At the center of the picture the end of a follicular capillary opens out like a fan and its mural endothelium becomes continuous with the pulp reticulum of the marginal zone. This same capillary termination is shown in a drawing in Fig. 13.

### Plate 43

Fig. 3 (Photomicrograph). — Arterial capillaries of the marginal zone in rabbit's spleen, distended and fixed in the living animal. The section has been stained with hematoxylin-eosin. The largest dilated vessel at the top is surrounded by a somewhat indistinct Schweigger-Seidel sheath. The capillaries below it come off as a single trunk in the third section away from this one. The open capillary extending to the left presents three branches, the

first one indistinct and without lumen in this section, extending upward a little to the left of the larger trunk. The second branch, just to the left of this, shows a distinct lumen for a short way but then passes out of the section. The third twig continues downward and to the left to terminate in a distended ampulla which is rather indistinct. Other capillary branches extend to the right in the picture, most of them terminating in the marginal zone about the follicle seen at the bottom of the picture.

FIG. 4 (Photomicrograph). — Centripetal arterial capillaries of the marginal zone in the human spleen, from same spleen as Fig. 3; section stained with phloxine-azure B. The thick Schweigger-Seidel sheath suddenly fades out as the vessel enters the marginal zone of the follicle where it divides and terminates in two ampullae, the upper one being distinct in this section. This photograph was shown before the Albany meeting of the American Association of Pathologists and Bacteriologists on April 3, 1926. The colored drawing of Fig. 14 represents a part of the same field.

PLATE 44

FIG. 5 (Photomicrograph). — Arterial capillaries of the pulp cords in rabbit's spleen, distended and fixed in the living animal. The follicle with its dilated artery is seen at the right. Arterial capillaries, venous sinuses and pulp cords are easily distinguished. Hematoxylin-eosin stain.

FIG. 6 (Photomicrograph). — Capillary of the pulp cord in rabbit's spleen, almost complete in a single section. Hematoxylin-eosin stain.

PLATE 45

FIG. 7 (Photomicrograph). — Section next in series to that of Fig. 6, showing termination of the same pulp capillary, with a well defined minute channel leading from the dilated ampulla into the adjacent venous sinus, the channel blocked by an endothelial cell which is about to escape from it into the venous sinus.

FIG. 8 (Camera-lucida drawing). — The same ampulla shown in Fig. 7, showing more clearly the detail of the rod-like endothelial cells lining the terminal arterial capillary, the pulp reticulum, a large phagocyte with ingested erythrocyte at lower right corner and, somewhat indistinctly, the wall of a venous sinus. The connection between ampulla and venous sinus is extremely narrow and blocked by an endothelial cell which has ingested an erythrocyte. The channel cannot be found in adjacent sections of the series. Length of the scale line represents 50 microns.

PLATE 46

FIG. 9 (Photomicrograph). — Arterial capillary of pulp cord of human spleen; from same spleen as Fig. 3; section stained with phloxine-azure B. The capillary is cut longitudinally for a long distance. In adjacent sections it was easily traced back to an arteriole with thick Schweigger-Seidel sheath. At its termination there is slight dilatation of the lumen to form an ampulla and the pulp spaces at either side are greatly distended. It does not open into a venous sinus. This photograph was shown at the Albany meeting of the American Association of Pathologists and Bacteriologists on April 3, 1926. The ampulla and adjacent structures are shown in colored drawing in Fig. 15.

PLATE 47

FIG. 10 (Camera-lucida drawing). — Arterial capillary of pulp cord in spleen of rabbit; serial sections at 3 microns, stained with hematoxylin-eosin; first of three sections in series. (1) nucleus of endothelial cell appearing also in the next section, designated to permit ready orientation; (2) nucleus of endothelial rod cell forming part of the wall of the terminal ampulla of arterial capillary, also appearing in part in the next section; (3) phagocytic pulp cell, also in the next section; (4) large nucleus recognizable also in the following section; (AC) arterial capillary; (DIA) diapedesis of nucleated wandering cell; (F) floor of a venous sinus; (P) pulp; (V) venous sinus Length of the scale line represents 50 microns.

FIG. 11 (Camera-lucida drawing). — Section next in series below that of Fig. 10. The terminal ampulla of the arterial capillary is almost complete in this section. (1) (2) (3) (4) nuclei facilitating orientation with Fig. 10; (11) nucleus appearing also in the next section, designated to assist orientation; (AC) arterial capillary; (F) floor of venous sinus; (P) pulp; (Ph) phagocytic cell; (V) venous sinus. Length of the scale line represents 50 microns.

PLATE 48

FIG. 12 (Camera-lucida drawing). — Third section of the series, next below that of Fig. 11. The site of the terminal ampulla is here hardly distinguished from ordinary pulp reticulum. (11) nucleus for orientation with preceding section, Fig. 11; (AC) arterial capillary; (DIA) wandering cell in diapedesis; (P) pulp; (Ph) phagocyte; (R) roof of venous sinus appearing for the first time; (V) venous sinus. In this series of three sections enough has been included in the drawings to indicate a distinct layer of pulp all about the terminal ampulla separating it from neighboring venous sinuses. The fenestrated character of the wall of the ampulla and of the walls of the venous sinuses is distinctly evident. Length of scale line represents 50 microns.

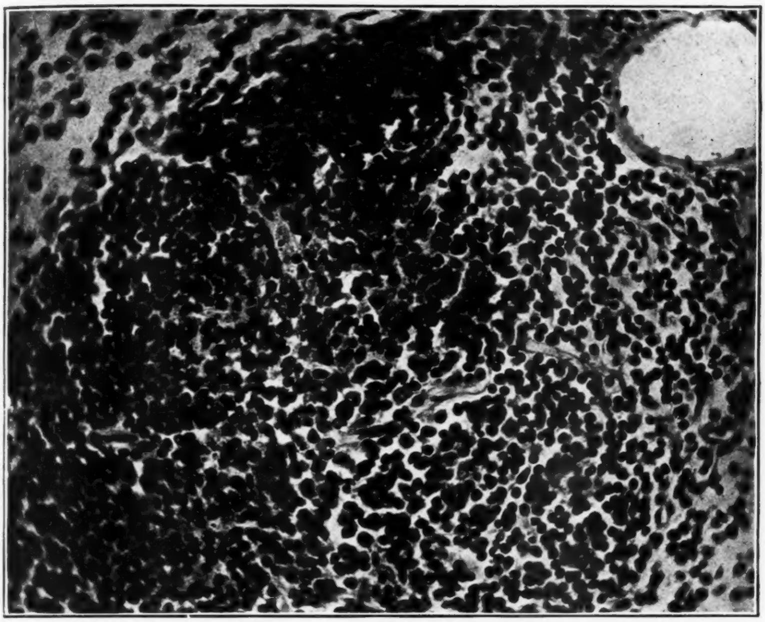
FIG. 13 (Camera-lucida drawing). — The identical terminal ampulla of follicular capillary of human spleen shown in Fig. 2. The drawing shows more distinctly the narrow lumen, the slender rod-shaped endothelial cells of the capillary wall going over by insensible gradations to the reticular pulp cells of the marginal zone. In adjacent sections of the series one can trace this vessel back to the center of the follicle. Length of scale line represents 50 microns.

FIG. 14 (Camera-lucida drawing). — The identical terminal capillaries of marginal zone of human spleen shown in Fig. 4, where the relation to spleen follicle can be ascertained. The detail of capillary wall and of pulp reticulum is more distinct in this drawing. The upper ampulla is directly continuous with the capillary lumen. The lower capillary is more irregular at its termination and what appears to be ampulla here is seen, on closer study, to be a dilatation located in the pulp at one side of the capillary termination. It would appear that the variable state of contraction of the mural endothelium at the moment of perfusion may be responsible for variations of this sort. Length of scale line represents 50 microns.

PLATE 49

FIG. 15 (Camera-lucida drawing). — The identical ampulla of pulp capillary of human spleen shown in Fig. 9. Detail of the capillary wall, of pulp reticulum and of wall of venous sinuses is somewhat more distinct here than in the photomicrograph (compare Fig. 10). Adjacent sections in the series reveal no direct pathway from ampulla to venous sinus. Length of scale line represents 50 microns.

FIG. 16 (Camera-lucida drawing). — Follicle, marginal zone and adjacent pulp of rabbit's spleen into which a suspension of bird's erythrocytes was introduced by injection into short gastric branch of splenic artery during life, followed one minute later by perfusion with Locke's solution and Helly's fixing solution to produce prompt fixation in the distended state. Note that the nucleated erythrocytes are abundant in the pulp spaces of the marginal zone and are escaping thence into the venous sinuses through the stomas of Mollier. This drawing and the microscopic field represented by it were both demonstrated before the American Association of Pathologists and Bacteriologists and the International Association of Medical Museums at Albany, New York, on April 3, 1926. (F) follicle; (FA) follicular artery filled with erythrocytes of rabbit; (MZ) marginal zone; (P) pulp cord; (Ph) phagocytic cell; (V) venous sinus; (1) avian erythrocyte passing through sinus wall from pulp of marginal zone; (2) avian erythrocyte sharply constricted at its middle in passage through wall of venous sinus. Length of scale line represents 100 microns.



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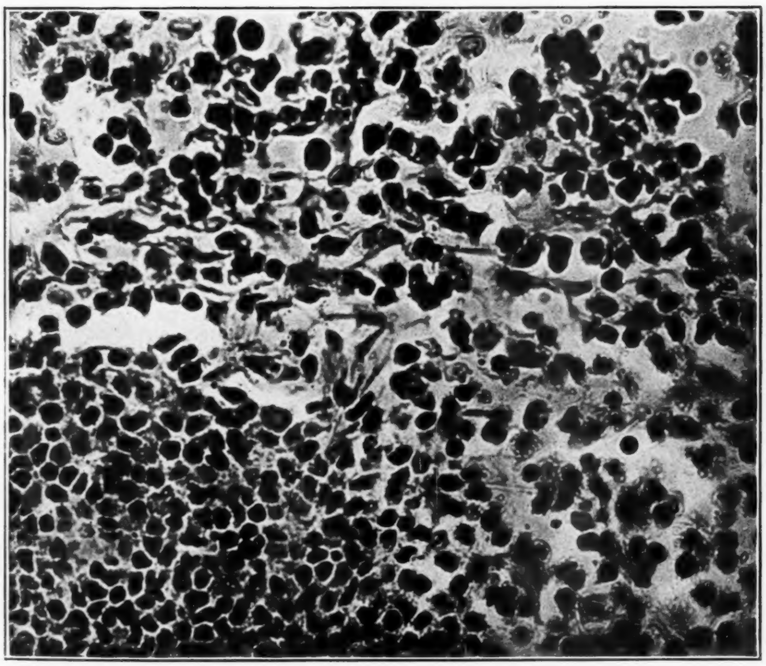

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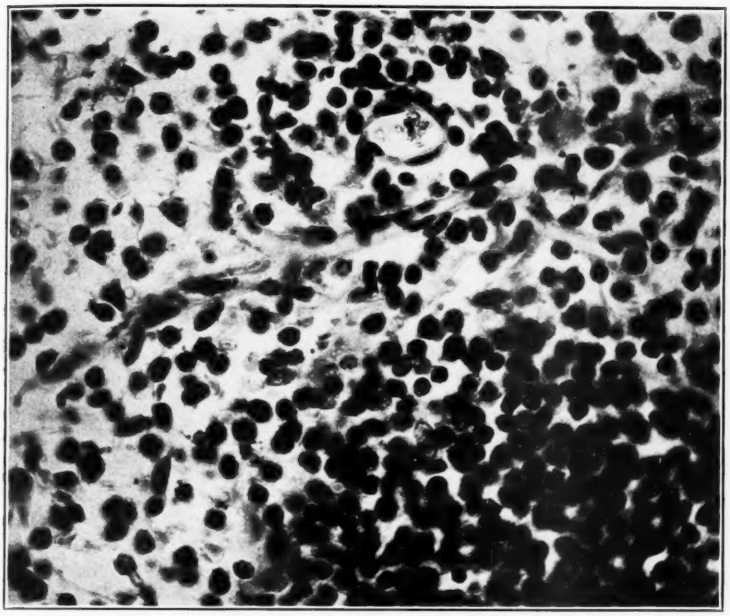
MacNeal, Otani and Patterson Finer Vascular Channels of Spleen



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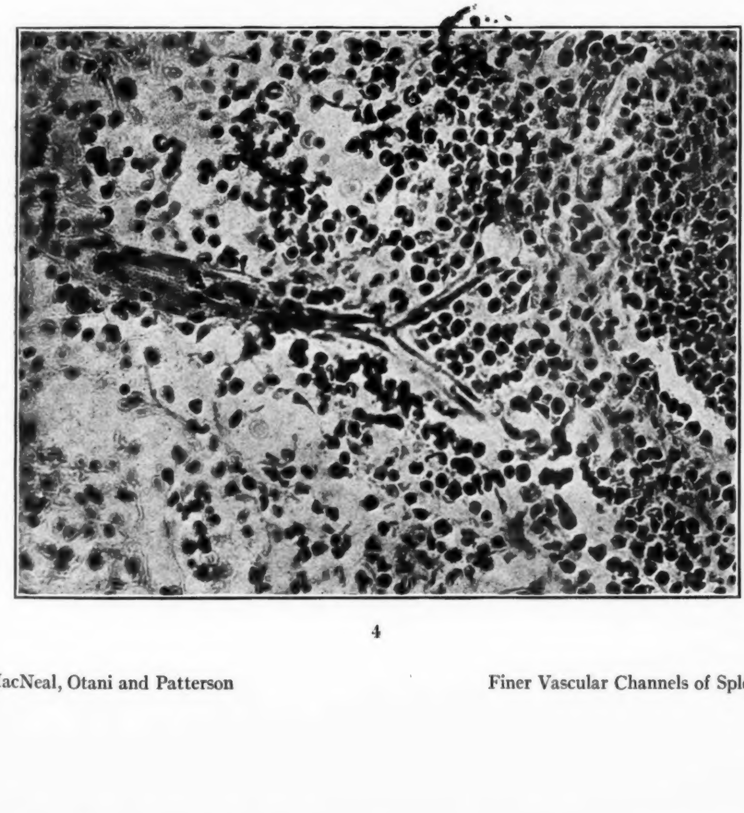

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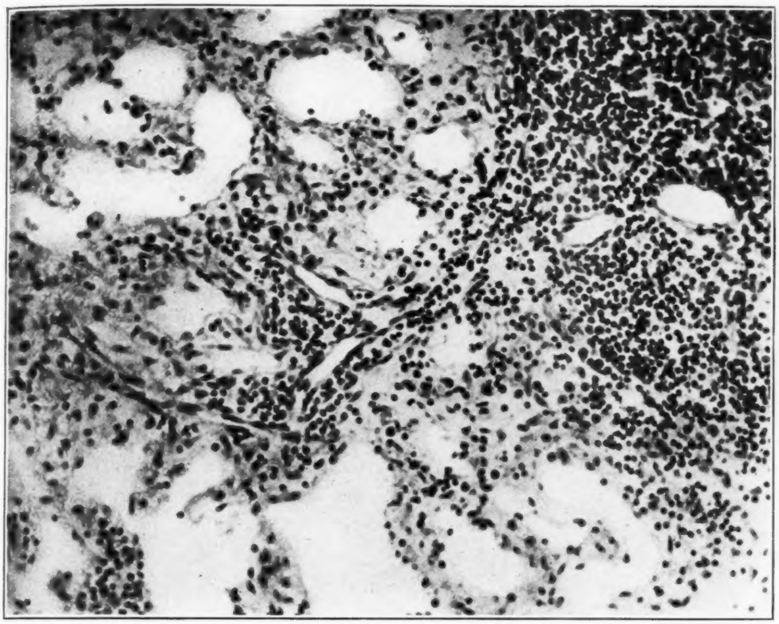
MacNeal, Otani and Patterson Finer Vascular Channels of Spleen



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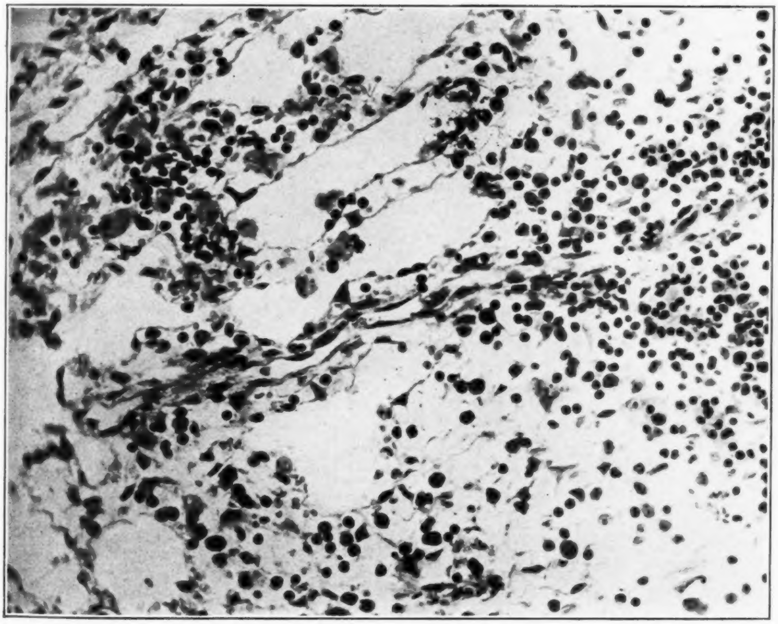

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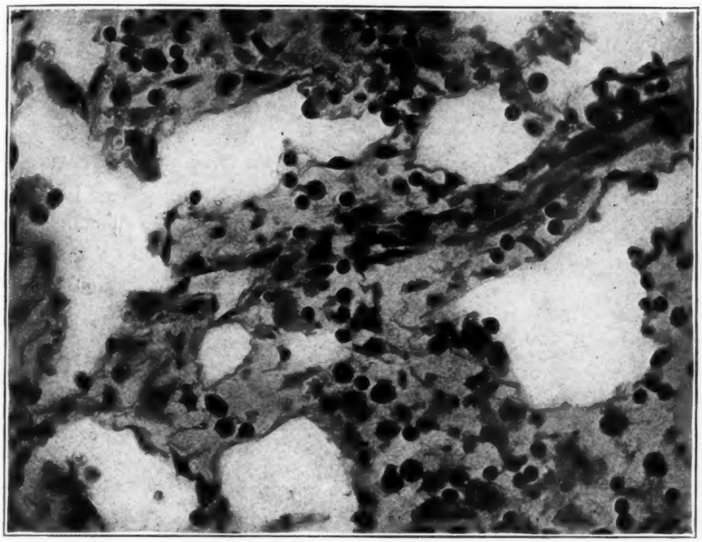

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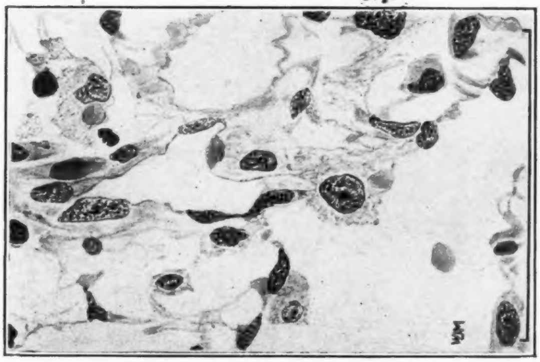

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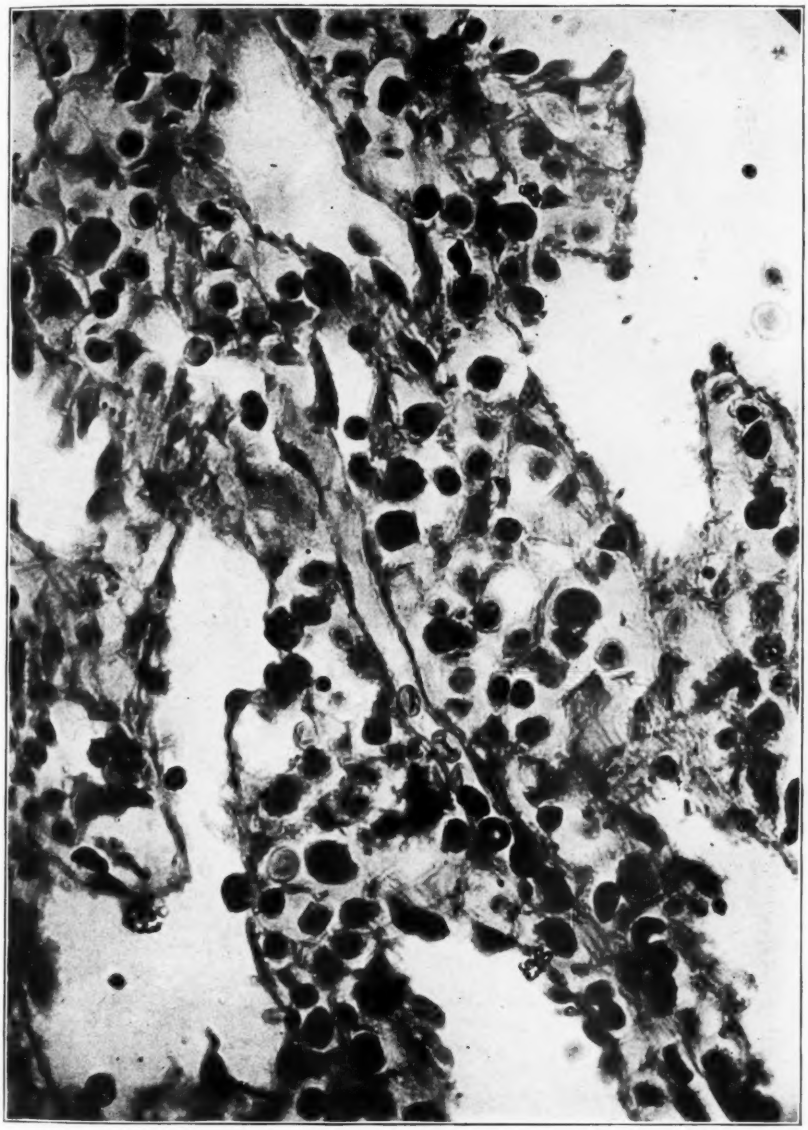

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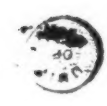
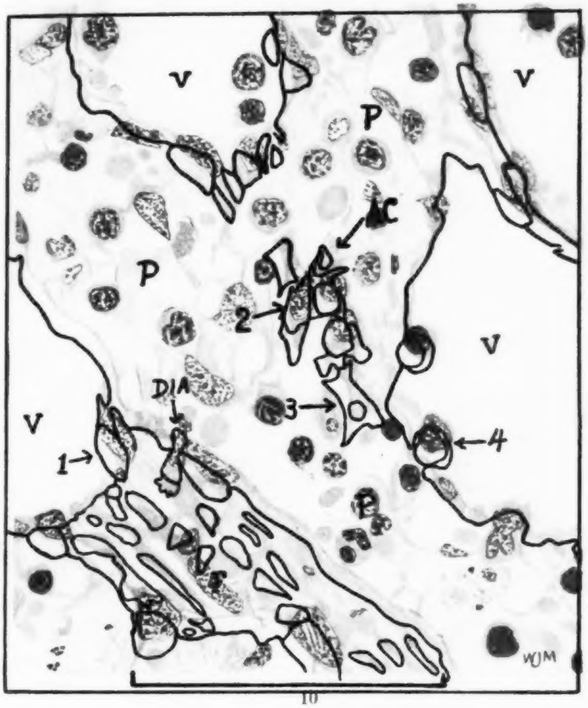
MacNeal, Otani and Patterson Finer Vascular Channels of Spleen



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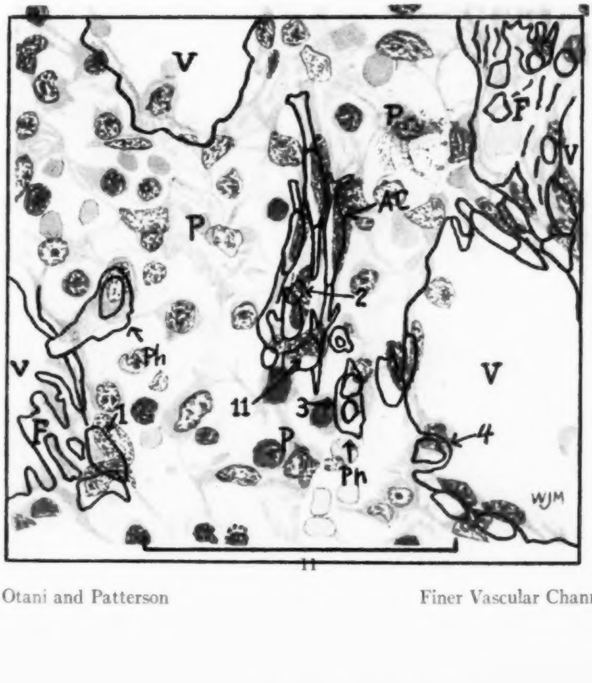


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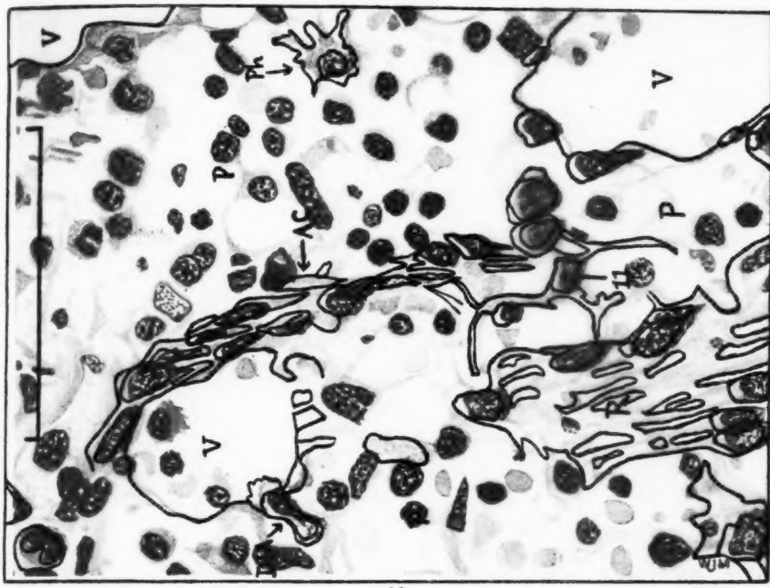

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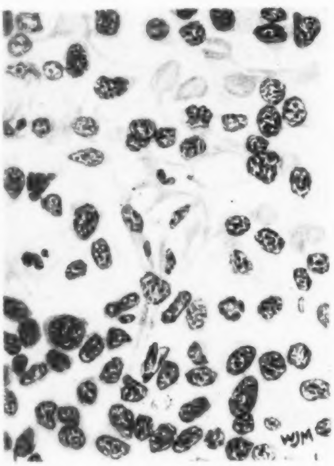

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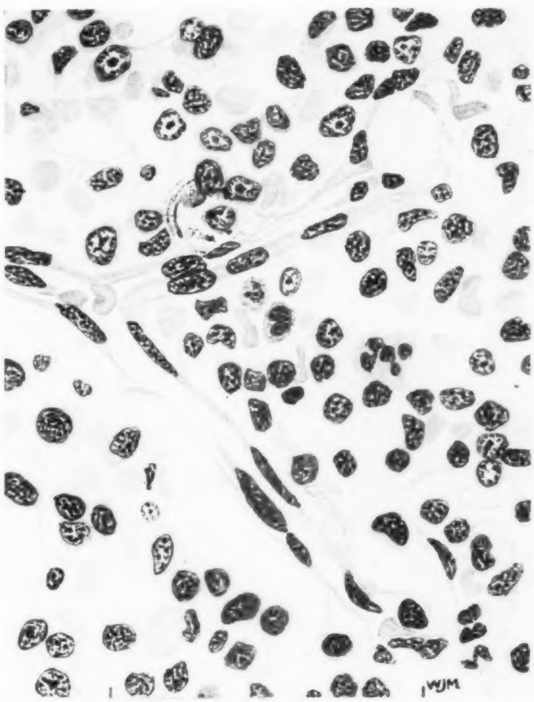

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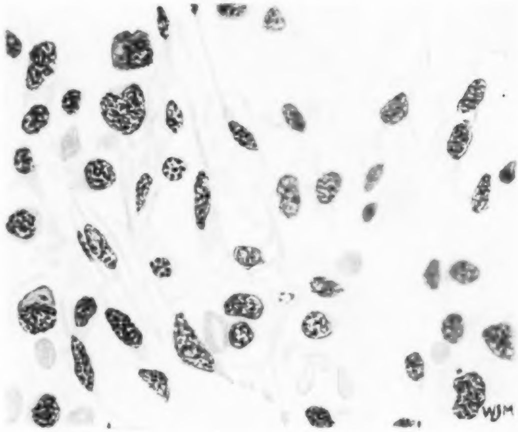

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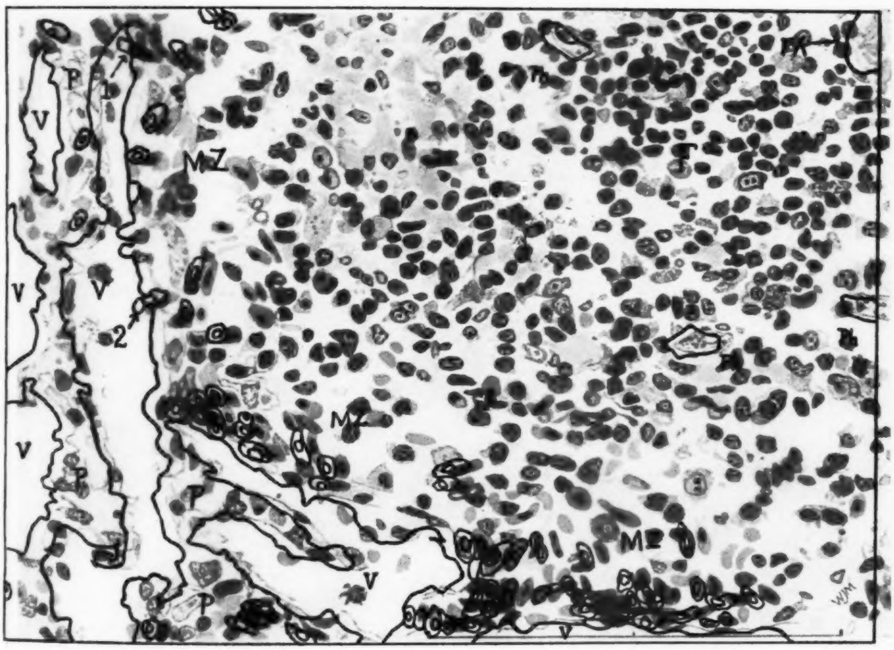


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# STUDIES ON THE ISLANDS OF LANGERHANS IN THE HUMAN PANCREAS*

## II. SIGNIFICANCE OF VARIATIONS IN STRUCTURE

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In a previous paper,[16] we have concluded that the islands vary in structure: some islands are connected with acini or ducts, while others are separated from the rest of the pancreatic tissue. After having established this morphologic fact we now intend to discuss the significance of these variations.

### 1. Islands Connected with Acini

That the majority of the islands are connected with acini without intervening fibers has been clearly demonstrated in our previous paper. However, whether such connection really means a transition from acinus to island or *vice versa*, as interpreted by Laguesse,[10] Gellè,[4] Vincent and Thompson,[23] Seyfarth[19] and others must be considered more carefully, because even Weichselbaum and Kyrle[24] and Opie,[15] who believe in an anatomic independence of the islands, admit an occasional break in the limiting fibers around the islands; so did Bensley[1] express his view quite emphatically that such connection does not necessarily mean transition between the two cell groups, though he assumes that the majority of the islands are in direct contact with the acini. Since Bensley's work has been done with the greatest care, many investigators accept his opinion. In the most recent literature Macleod[13] maintains that acini and islands are as distinct and separated from each other, both anatomically and physiologically, as are the anterior and posterior lobes of the pituitary glands.

As a matter of fact, no sufficient proof has as yet been offered by any of the previous workers which would settle the question of the anatomic relation of the islands to the acini.

If one examines a great many specimens of pancreas, occasionally one can find islands of unusually large size; such islands also are seen

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in the pancreas of infants. Even in the same section there can be recognized islands of exceedingly great variety in size. Therefore, if one finds an occasional large island among the other ordinary ones in a certain pancreas, one cannot maintain that this condition represents a hypertrophy of the islands.

If one finds, however, great numbers of unusually large islands in a pancreas, one may consider them enlarged. Koch,[9] Lang,[11] Cecil[2] and Warren[25] have reported cases described by them as adenoma of the islands. Nuboer,[14] Martius,[12] Hertel[6] and Gray[5] have also described cases of hypertrophy. Seyfarth[19] described a case of hypertrophy in the pancreas of a child which died of inanition.

We have also observed a pancreas in which very large islands were found throughout the organ, while other specimens showed exclusively small islands.

Accordingly, there is no doubt that a process of hypertrophy of the islands may occur under certain conditions. However, whether such enlargement does occur by transition of the acinar cell or by hyperplasia of the island cell proper, is still under discussion in the literature. We believe that knowledge of the histogenesis of the enlargement may contribute to the decision of this question. Therefore, we shall first discuss this point in our attempt to come nearer to the solution of this problem.

The enlarged islands generally show the following characteristic features:

(*a*) They are exceedingly irregular in size and show no complete limiting fibers around the islands; they usually are connected with the acini at the greater part of their circumference. (Fig. 1.)

(*b*) In the periphery of the island between the insular and acinar cell groups there are found fine fibers having no relation to the capillaries in the islands, which by their location, however, appear to be remnants of the basement membrane of the acini. (Figs. 1 and 2.)

Fig. 1 shows one example of these relations; we believe that this island is in a process of hypertrophy. The fine fibers are located especially at the periphery of the island and in the center there are rather thick fibers enclosing capillaries, which apparently were present in the island before its enlargement. The fine fibers are derived from the basement membrane of acini, which is proved by serial sections and also by the fact that they have no relation to the capillaries within the island tissue.

(*c*) At the boundary of these islands there are found various changes in cell elements; these are observed in the hematoxylin-eosin preparations (Figs. 3 and 4). The cell groups which apparently belong to the acini, stain neither with hematoxylin nor with eosin distinctly. They do not show basophile or zymogen granules. They contain central nuclei but they are different from the insular cells. In short it cannot be pointed out to which cell group they belong.

These findings are more clearly demonstrated in the preparations stained with neutral gentian violet; in the same acinus two cell groups can be found which differ from each other. Besides there is a third type which does not stain with this dye distinctly.

These three findings alone already suggest that the enlargement of the islands occurs mainly by transformation of acinar cells into island cells, not alone by hyperplasia of island cells proper, because mitosis of insular cells within large islands has been found very rarely; therefore, we may infer that such occurrence is not necessarily a characteristic feature of enlarging islands. Naturally our negative result would not necessarily exclude the possibility of a hyperplasia of island cells. However, we can advance other arguments against such a view.

If enlargement occurs by hyperplasia of the insular cells only, we should be able to observe evidence of pressure by the peripheral cell groups of the very large islands on the neighboring acinar tissue. These findings have never been observed in our material. These relations are demonstrated in Figs. 1 and 5. Furthermore, sometimes we have seen a form of large island which consists of a dumb-bell-like fusion of two islands (Fig. 5). The enlarged islands often show unusual irregularity; they protrude into the acinar structures like the arm of an octopus (Fig. 1 and also see Fig. 14 in the previous paper [16]). We would be unable to interpret these findings if we accept the enlargement as being caused by hyperplasia of insular cells only.

Fig. 6 shows the periphery of a very large island. In the center there is a peculiar glandular structure consisting of island cells. Its lumen contains a fluid which can be recognized as identical with that in the ducts, because it stains with eosin very deeply and in the next serial section stained with neutral gentian violet, this fluid shows a light brown color, while the blood in the arteries is deep brown. The sections from this pancreas show markedly dilated

ducts and terminal ducts. From its location in this large island we believe that this glandular structure is in a stage of conversion from acinar cells to island cells.

It is common in the adult pancreas to find peculiar altered cell structures within the acini which stain very dark with hematoxylin and are spindle-shaped (Fig. 6). The same cells are found within the very large islands which evidently are in a process of enlargement, especially at the periphery. Figs. 6 and 7 represent typical examples.

Fig. 7 is a section from a pancreas which shows a great number of unusually large islands in every portion. This picture was made from the periphery of a very large island, where such dark cells differing from capillary cells are seen.

Fig. 8 shows an irregularly shaped island. In the lower portion there is an insular structure indicating the original island, the other light cell groups representing newly formed island structures which can be interpreted as being limited by the basement membrane of the acini on account of the presence of the acinar cells between the light cells. Some of these basement membranes contain capillaries. This picture as well as Fig. 7 indicates the histogenesis of capillary formation in the growing islands. The basement membranes of the acinar cell groups, which contain a capillary, remain in the enlarging island as part of its capillary system.

The fact that peculiar homogeneous cells appear often among the acinar structures in the adult pancreas has been described by Opie,[15] Weichselbaum and Kyrle,[24] and Fahr.[3] Opie described these cell groups as altered acini, the lumen being usually very conspicuous, often dilated. We observed, however, the homogeneous cell groups without any remarkable dilatations of their lumen and frequent hyperplasia of these cell elements. Fig. 9 shows a group of these homogeneous cells.

Fig. 10 shows the same cell group with a mitotic figure of a centroacinar cell. An acinar cell has lost its basophilic staining reaction and apparently represents a transitional stage between the acinar and homogeneous cells.

Fig. 11 demonstrates the homogeneous cell in groups. Among these a few acinar cells are still present. Though no distinct cell division figure can be seen in this section, there undoubtedly exists a process of hyperplasia of homogeneous cells. In this pancreas numerous large islands are found.

Fig. 12 illustrates the fact that the homogeneous cell groups may also occur at the periphery of the very large islands. In Fig. 13 one sees several homogeneous cell groups which differ somewhat from the picture described above. They show various stages of transition from acinar to homogeneous cells.

Fig. 14 shows a similar picture. Many of the homogeneous cells stain with eosin very intensely. Among these there are several very dark cells deeply stained with hematoxylin, which often can be found in the acinar structures. This picture also shows various stages of transformation of the homogeneous cells. The structure of this cell group is almost identical with that of an island; however, it cannot be classed as a true island on account of a small number of dark cells surrounding the homogeneous cell groups which appear to be acinar cells by their morphologic characteristics. Inasmuch as this island-like cell group is bordered by rather thick connective tissue, which is proved by serial sections, these dark cells do not belong to the neighboring acini.

The appearance of the cell groups, presented in Figs. 9 and 10, is not exactly identical with that of the island, although it resembles the latter in general makeup. Figs. 13 and 14 come even nearer to the composition of the island. On account of the presence of hyperplastic processes among these homogeneous cell groups and also because at present we cannot distinguish the homogeneous cells from those of the islands, it is reasonable to assume that these cells represent a certain stage in the transformation of acinar cells into island cells, *i. e.*, that they are the actual island-builders. Their occurrence at the periphery of very large islands and also in pancreases that contain numerous very large islands supports this view strongly.

The island cells are composed of so-called A and B cells whose granules stained by Lane's method are distinct from those of the acinar cell. This fact was advanced as an argument, especially by Bensley, Macleod and others, in favor of the different structure of the two cell groups, and led to their conclusion that no transition is possible from one to the other. However, these granules (A and B) are not present in every island.

Fig. 15 shows dark cells which are comparable to A cells stained with neutral gentian violet. The dark cells are located chiefly at the periphery of the insular cell groups; some of them are arranged in acinus-like structure in the center of which several clear cells are

situated, suggesting proliferated centroacinar cells. Their location and our previous observations presented in Figs. 10 and 11 support this explanation.

In silver preparations which were kept in 2 per cent silver nitrate solution over 100 hours in the incubator, fine brown or deep brown granules occur in the island cells, while the acinar cells remain clear (Fig. 16). The same granules, however, also occur in a small number of acinar cells, as well as in the duct cells which have no relation to the island; they appear isolated between acinar cells or duct cells (Figs. 16 and 17). These argentophilic granules within the acinar cells have been observed in the frog pancreas by Saguchi.[20] We have also found similar granulated cells within acini in preparations stained by neutral gentian violet. This brings us into accord with the opinion of Vincent and Thompson [23] and others who state that insular cells are occasionally scattered within acinar structures. These findings are an indirect argument in favor of transformation of acini to island cells.

After demonstrating on the basis of our morphologic studies that acinar cells are able to change into insular cells, it is logical to concede that the enlargement of the islands takes place by a process of transition of the acinar cells. The interpretations of Opie,[15] Weichselbaum and Kyrle,[24] Helly,[7] Bensley,[1] Ukai [22] and others who claim that such does not happen, cannot refute our conclusions, because they never as yet have given such clear-cut findings as ours and moreover our conclusions are based upon the findings, described in our first paper, that most of the islands are connected with acini, without intervening fibers.

Whether the reverse may be possible, namely, changing of island cells into acinar structure, as maintained by Laguesse,[10] Seyfarth [19] and others, is difficult to confirm. Certain facts, however, indicate its occurrence. Among numerous specimens we have found specimens of pancreas which contain small islands throughout (Fig. 18), whereas others showed very large ones (see Fig. 14 in first paper [16]). In the former type of pancreas the numerous islands still show direct connections with acini and the border of both cell types represents a very gradual transition without any discernible signs of degenerative changes in the island cells. Such pictures could be interpreted as transition from island to acinar cells. The fact that the islands actually present varying structures as described in our first paper,

favors this view indirectly. Since the acinar cells do change into those of the islands, the reverse process might occur.

Schmidt [21] and others stated that transition might occur under certain pathologic conditions only. Since we have very often found enlarged islands in normal pancreas and, furthermore, recognized the connection of islands with acini as of common occurrence, we are of the opinion that transition may occur under normal conditions. But though there are some islands connected with acini that do not show distinct morphologic transition, we think both are genetically related, since the infant pancreas contains islands connected with acini more often than the adult pancreas.

## 2. Islands Connected with Ducts

In the previous paper we maintained that the islands connected with ducts may be classed in two subgroups: (*a*) The area of continuity between island and duct is very narrow; and (*b*) the duct and islands are in broad communication.

The former type, shown in our previous paper Figs. 5 and 6, has often been found in the pancreas of infants, especially in new-born individuals, and can be interpreted as persistence of an embryonal structure, in the same way as undifferentiated glomeruli are found in the cortex of the kidney, occasionally even in children of six or seven years. The picture in the congenital syphilitic pancreas supports this explanation. If all acinar cells at the periphery of an island connected with acini become transformed into island cells, such an island may remain connected with the terminal duct. Fig. 19 exemplifies such occurrence most strikingly. Therefore, islands communicating with ducts over narrow strips will also be interpreted as resulting from such transitions of acinar cells.

However, the islands connected with ducts at a wide circumference differ from the former type. They have been found in adult pancreases and never in those of infants. This leads us to believe that they are not embryonal remnants (see Fig. 8 in the first paper[16]). One-half of the epithelial lining of a duct does not show true duct-cell character; the cells are rather similar to those of the islands; such cell groups have no basement membrane dividing them from the island cells. Islands of this type are usually small and show an undifferentiated structure. We assume from such findings that the

duct cells have a definite relation to the new formation of the islands. Fig. 20 demonstrates a changed duct.

Bensley recognized the occurrence of an interstitial growth of islands from duct cells in guinea-pigs. We also made similar observations in human pancreas.

A peculiar proliferation of duct epithelium due to unknown conditions was described by Priesel[17]; our material presents a certain number of such instances. We found such proliferation in oval or round island-like forms and proved with the aid of serial sections that these cell accumulations were connected with the ducts by a more or less narrow zone of cells. Though they resemble the island structure, they lack capillaries; therefore, they cannot be classed as true islands.

The claim has been made by Weichselbaum and Kyrle,[24] Helly[7] and Bensley,[1] that the duct cells possess the power of forming islands even in adult life. However, until now no definite proof has been offered in the literature to substantiate such statements. We feel that our findings established this fact definitely.

### 3. Islands Separated from the Rest of the Pancreatic Tissue

The fact that islands exist entirely separated from the rest of the pancreatic tissue and are connected with neighboring structures by blood capillaries only, is the most important factor in support of the theory of internal secretion. However, no definite morphologic proof has been brought forward for the human pancreas to show that the islands are entirely independent structures.

The separate islands we found are limited by fine fibers. Judged by their histologic appearance and micro-chemical proofs, they are normal in every way. Kirkbride[8] found that islands remaining in the pancreas which becomes fibrosed after ligation of its ducts still show the typical so-called A and B granules. This indicates that, if the islands have some physiologic function, this particular type might show the same activity. Some islands are connected with the rest of the pancreatic tissue by blood capillaries only, which in addition to above considerations leads to the conclusion that they have an internal secretory function in human pancreas. They do not represent, however, an independent organ *sui generis*, because normally not all islands show an independent structure.

### 4. New Formation of the Islands in the Adult Pancreas

That the acinar cells are able to change into island cells is an undeniable fact. The homogeneous cells, intermediate in type between the acinar, centroacinar and island cells, not only occur in groups but a hyperplasia of these cells does exist. This supports the conception that a new formation of islands may occur by transformation of acinar and centroacinar cells in adult life.

We also assumed that the duct cells are capable of forming islands. Since the centroacinar cells belong to the duct in a broad sense,[18] there are really two elements which play a part in the formation of the islands, namely, acinar and duct cells. It does not seem plausible to have the islands derived from two different elements, *i. e.*, to accept a dualistic view with regard to their formation. However, if one examines the undifferentiated pancreas of the new-born, such can easily be conceived (see Figs. 4 and 7 in the previous paper[16]), because such islands are connected with acini as well as ducts; furthermore, the argentophilic granules can be found in the acinus as well as in the duct cells.

## Conclusions

1. The process of hypertrophy of the islands of Langerhans in the adult pancreas is mostly due to transformation and hyperplasia of acinar and centroacinar cells, the hyperplasia of the island cells proper being relatively insignificant.

2. The acinar cells are able to change into island cells. The reverse process might occur, though it is difficult to prove morphologically.

3. In this respect, the direct contact between islands and acini indicates a genetic relation.

4. One type of island connected with ducts is interpreted as a remnant in the postembryonal pancreas or as resulting from the transformation from acinus to island cells, while the other type has a definite relation to the new formation of islands in the adult pancreas.

5. The fact that islands entirely separated from the rest of the pancreatic tissue do exist under normal and pathologic conditions may be regarded as morphologic evidence of an internal secretory

activity of the islands. They cannot be accepted, however, as constituting an organ *sui generis*, since normally most of the islands are connected with acini which are capable of changing into island cells.

6. The new formation of the islands in adult pancreas may occur by transformation and hyperplasia of the acinar, centroacinar or duct cells.

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## DESCRIPTION OF PLATES

Preparations illustrated in Figs. 1, 2, 3, 5, 16, 17, 18 and 19 were made by the silver impregnation method, followed by hematoxylin-eosin. All others except Fig. 15 were stained with hematoxylin-eosin.

### Plate 50

Fig. 1. Case 16, 27 years. Insufficiency of mitral valve. A very large island. In the lower portion there is a peculiar structure like the arm of an octopus. This is part of an adjoining island. × 140.

Fig. 2. Case 16. This picture was taken from the periphery of a very large island, showing fibers not containing capillaries. × 500.

Fig. 3. Case 17, 52 years. Pneumonia. This island shows gradual transition into acini, especially the upper portion. × 500.

Fig. 4. Case 75, 35 years. Endocarditis. Cell changes at the connection of island and acini.

### Plate 51

Fig. 5. Case 72, 3 years. Pneumonia. Section was cut from the head of this pancreas. A dumb-bell-like fusion of two islands. × 140.

Fig. 6. Case 49, 63 years. Carcinoma of the common bile duct. The picture demonstrates the periphery of a very large island, showing glandular structure of island cells (in the center), dark cells within the acinus and dark cells within the island. × 500.

Fig. 7. Case 17. Photomicrograph was taken from the periphery of a very large island, showing complicated cell changes; dark cells (in the center), capillary in the basement membrane of acini (upper part) and a cell group with lumen suggesting a terminal duct (lower portion slightly to the right). × 600.

FIG. 8. Case 73, 71 years. Hemorrhage of middle cerebral artery. Exceedingly irregularly-shaped island cell groups. The light cell groups in lower part indicate original island, in the upper part newly forming island. Dark cells are acinus cells. × 400.

PLATE 52

FIG. 9. Case 73. Homogeneous cells in group. × 550.

FIG. 10. Case 66, 30 years. Stenosis of mitral valve. Homogeneous cell group, showing a mitotic figure of a centroacinar cell. × 800.

FIG. 11. Case 17, 52 years. Pneumonia. Homogeneous cell group, within which few dark cells (basophilic) still remain. The dark cells are situated around the homogeneous cell group (compare with Fig. 15). × 550.

FIG. 12. Case 73. The picture was taken from the periphery of a very large island, which presents homogeneous cells in groups. × 500.

PLATE 53

FIG. 13. Case 73. The homogeneous cell groups, showing various stages of transformation from acinar cells. × 600.

FIG. 14. Case 73. An island-like cell group, containing few acinar cells. × 500.

FIG. 15. Case 8, 34 years. Peritonitis after perforated duodenal ulcer. An apparently newly forming island, showing dark cells, which are comparable to so-called A cells, located mostly around the light cells. Acinus-like structure is seen at the lower portion. Fixed in 20 per cent formalin, stained with Kultschitzky's hematoxylin after treatment with potassium bichromate. × 400.

FIG. 16. Case 56, 8 years. Uremia. Island cells contain argentophilic granules (lower portion), the acinar cells remain clear. An isolated argentophilic cell within the acinus (upper part). Section was kept in 2 per cent silver solution five days for impregnation and then stained with hematoxylin. × 800.

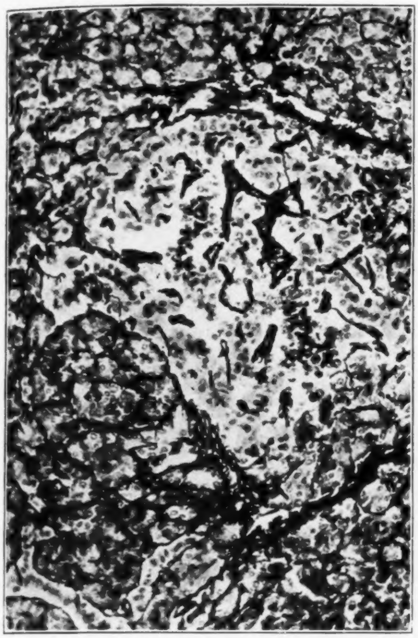
PLATE 54

FIG. 17. Case 56. A pancreatic duct cell showing argentophilic granules (in the center). × 800.

FIG. 18. Case 82, 27 years. Postoperative shock (tenorrhaphy of left hallux). This pancreas contains small islands connected with acini throughout. Both islands are cut at their widest diameter as seen on serial sections. × 120.

FIG. 19. Case 56. An island connected with the terminal duct. × 540.

FIG. 20. Case 77, 50 years. Carcinoma of bladder. An island-like structure, showing connection with duct. × 380.



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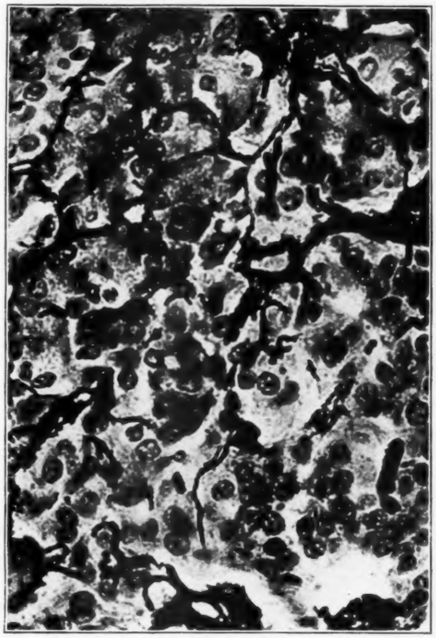

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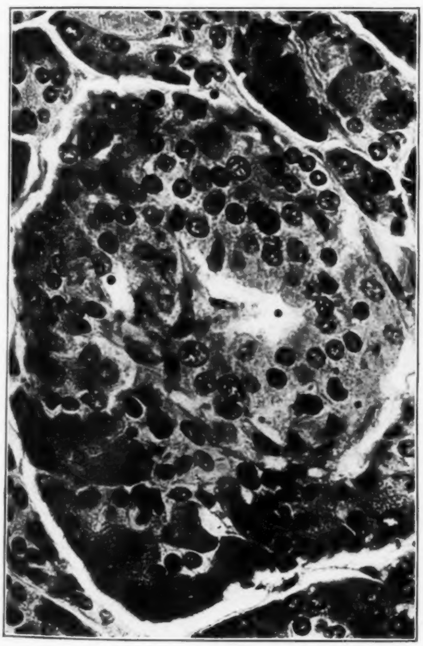

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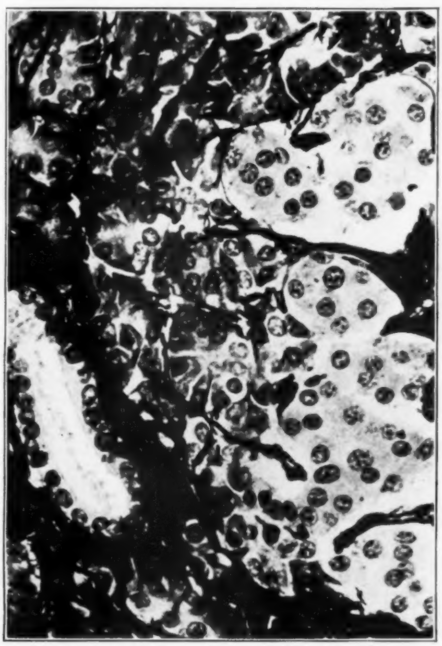

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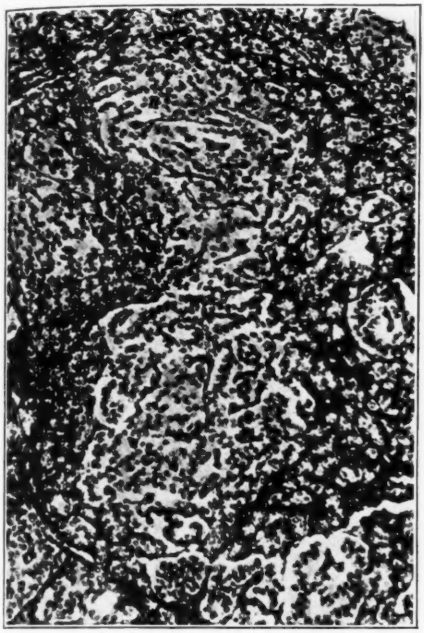
Otani

Islands of Langerhans in Human Pancreas



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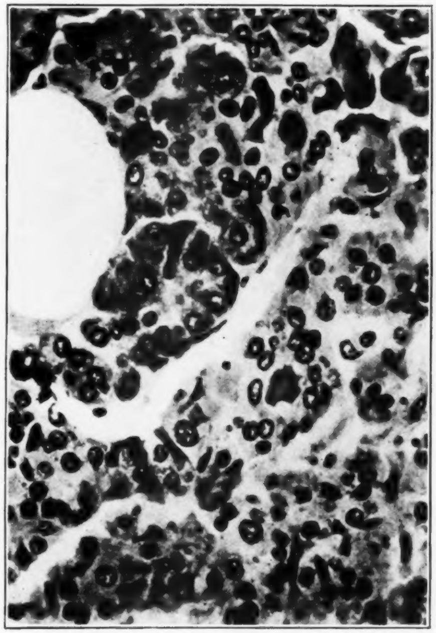

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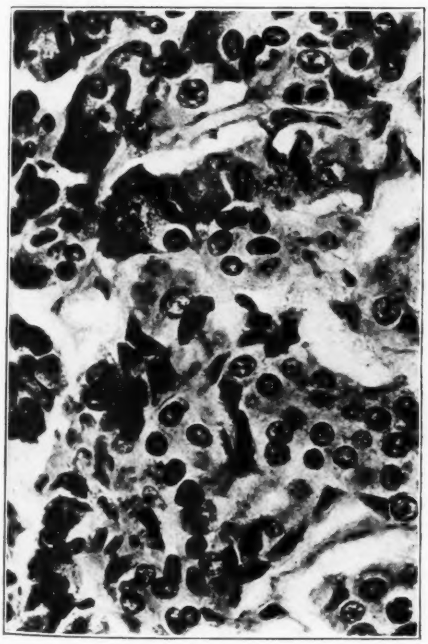

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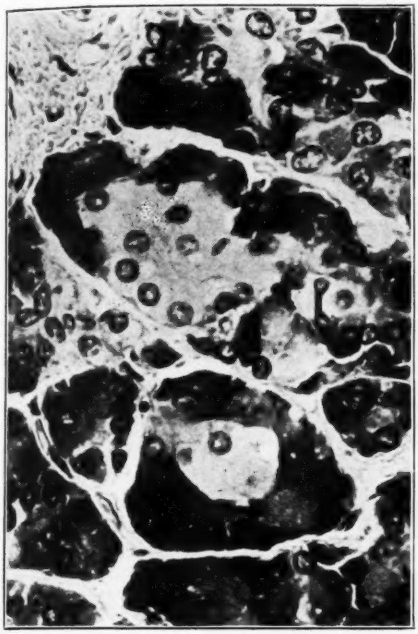
Otani

Islands of Langerhans in Human Pancreas



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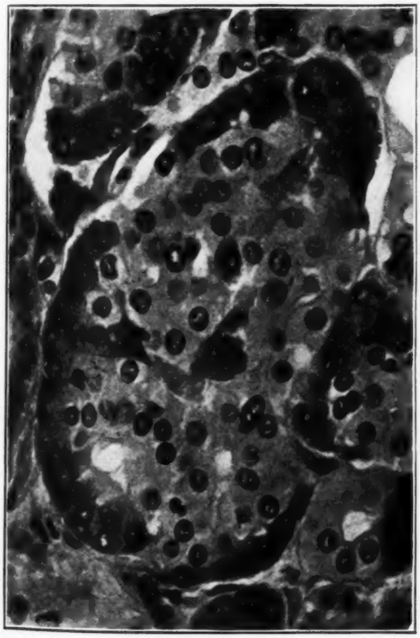

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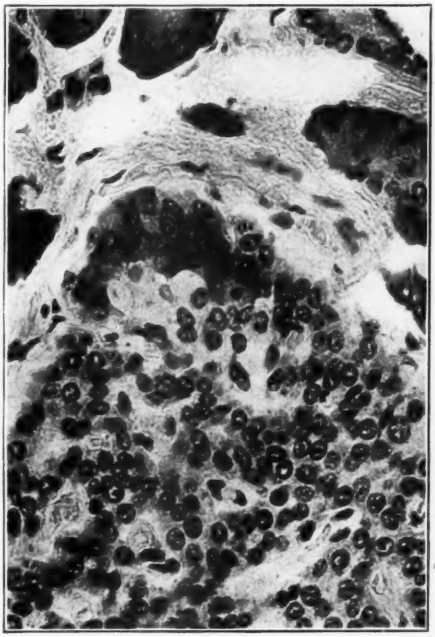

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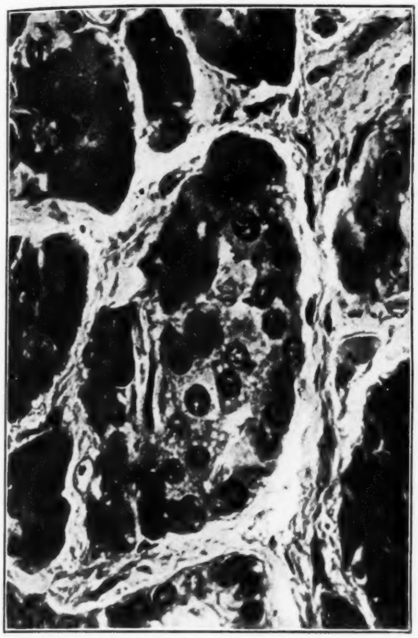
Islands of Langerhans in Human Pancreas



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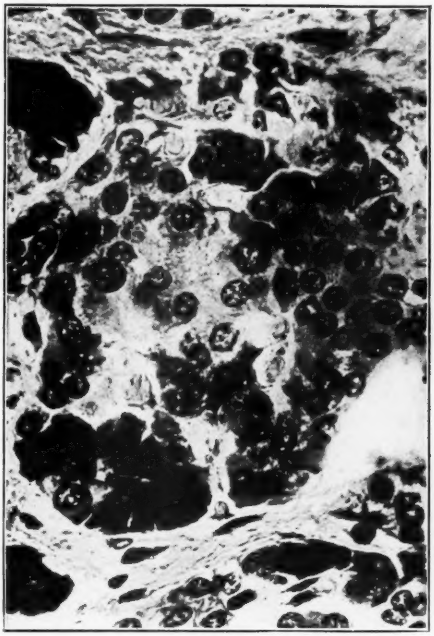

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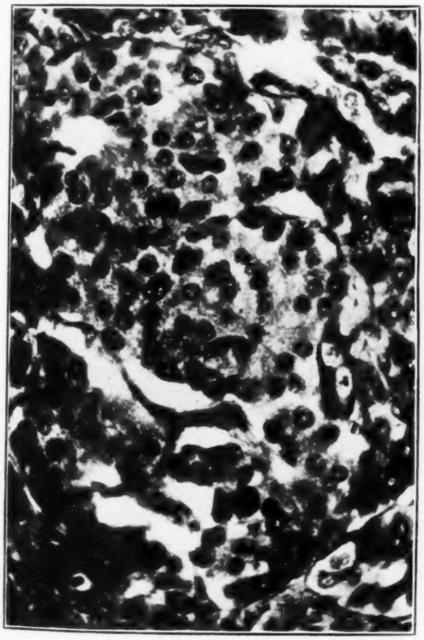

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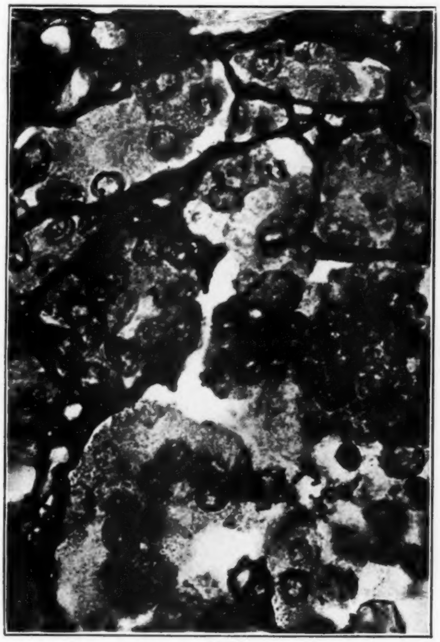

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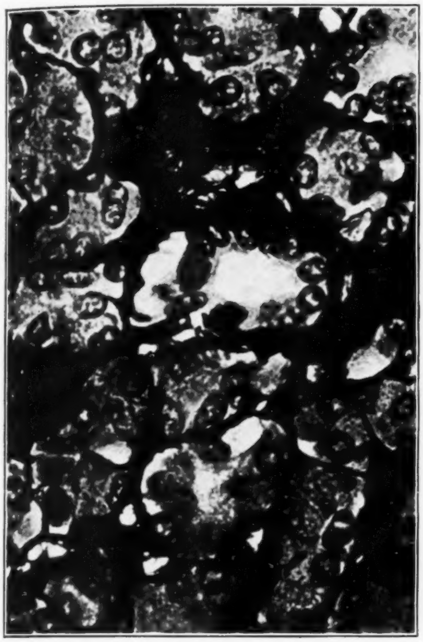
Islands of Langerhans in Human Pancreas



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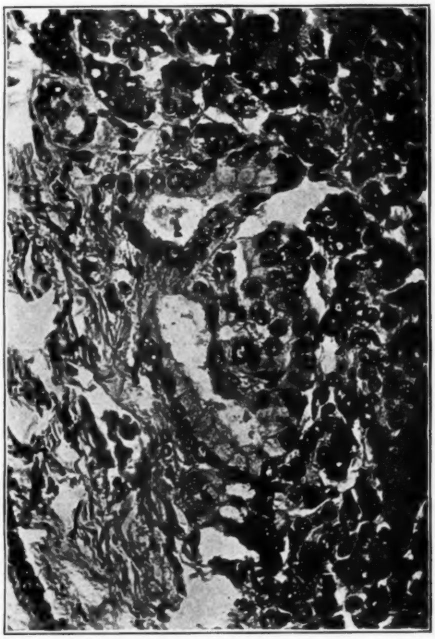

20

Otani

Islands of Langerhans in Human Pancreas

# A REPORT OF TWO CASES OF ESSENTIAL ADRENAL INSUFFICIENCY (ADDISON'S DISEASE)*

E. M. Medlar, M.A., M.D.

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Addison's disease is a clinical entity in that it represents a deficiency of adrenal function to a level incompatible with life. Associated with this adrenal insufficiency there is usually manifested a pigmentation of the skin and mucous membranes, languor and lassitude, hypotension and quite often a derangement of gastro-intestinal function. Extensive destruction of adrenal tissue is always present in Addison's disease but it is quite apparent that this destruction is not always caused by the same agent. From the pathology of the adrenal the cases have been divided (Bittorf) into two essentially different groups. The first group is that of essential or idiopathic adrenal insufficiency in which no infectious agent or tumor growth plays a part. The second group is that of secondary adrenal insufficiency and comprises all cases in which infections, such as tuberculosis, or tumor growths so derange the adrenal function as to cause, clinically, the symptom-complex originally described by Addison.

In a survey of the medical literature of the United States, all the cases of Addison's disease found recorded with necropsy findings fall into this second group. Warthin [1] in a paper on thymic hyperplasia briefly mentions three cases which belong to the first group. During the past year in the service of the Wisconsin General Hospital there have occurred two cases of essential or idiopathic adrenal insufficiency. The following is a brief clinical and necropsy record of these two cases.

## Histories of Cases

Case H. Male, age 28, single, Canadian. Graduate student in the College of Agriculture. Admitted to student infirmary April 27, 1926. Patient ill one week. Chief complaints were weakness, severe

* Received for publication November 10, 1926.

frontal headache, constipation, vomiting and anorexia. No abdominal pain. No chills or perspiration. Extreme thirst.

*Physical Examination.* Patient looks acutely ill. Skin has a distinct brownish tinge. There is marked cyanosis. Mouth dry. Tonsils large and succulent. Chest examination negative. Heart normal. Pulse rapid and regular. Blood pressure: systolic, 80 and diastolic, 48. Abdomen soft. Spleen and liver palpable. Skin dry and rough. Reflexes normal. Urine: albumen, trace. Blood: hemoglobin, 86 per cent; red blood cells, 5,330,000; white blood cells, 14,400; polymorphonuclears, 77 per cent; lymphocytes, 20 per cent; mononuclears, 3 per cent. Widal negative.

April 30. Patient apparently somewhat improved. No further positive findings. Blood pressure: systolic, 70 and diastolic, 36. Only positive findings are circulatory depression, pigmentation of the skin and dehydration. Urine: albumen, trace. Blood: white blood cells, 6,200; polymorphonuclears, 57 per cent; lymphocytes, 29 per cent; and mononuclears, 10 per cent.

May 2. Patient shows no improvement. Spinal puncture negative. Blood pressure: systolic, 73 and diastolic, 36. Urine: albumen, trace. Blood: hemoglobin, 74 per cent; red blood cells, 4,450,000; white blood cells, 16,900; polymorphonuclears, 65 per cent; and lymphocytes, 23 per cent. Blood culture negative. Vomitus contained some blood. Stool positive for occult blood.

May 3. Patient died.

*Clinical Diagnosis.* Addison's disease.

NECROPSY. Thirty-six hours after death. The essential findings of the necropsy are as follows.

*Thymus.* Weight 49 gm. Hyperplastic.

*Lymphoid Tissue.* Intestinal lymph follicles markedly enlarged. Mesenteric lymph nodes the size of almonds. The tonsils are large. Microscopic sections show hyperplastic lymphoid tissue.

*Skin.* Appears pigmented and dry.

*Adrenals.* The organs are small and firm. Microscopic examination shows marked atrophy of the cortex with large areas in which there is no adrenal parenchyma. There is marked fibrosis of the cortex and medulla. Extensive lymphocytic infiltration exists throughout the organ. Both organs present a similar appearance. No tuberculosis is found. Semilunar ganglia normal.

*Spleen.* Weight 270 gm. Measures 12.5×8×3 cm. The organ is

firm and dark red. The capsule is not thickened. On section the organ appears congested and the Malpighian corpuscles are very prominent. Microscopic examination shows congestion and hyperplastic lymphoid tissue.

*Testes*. Normal.

*Diagnoses*. Status thymico-lymphaticus; atrophy and fibrosis of adrenals.

CASE B. Female, age 29, housewife. Has always lived in Wisconsin. Entered hospital April 21, 1926. Chief complaint was weakness. There has been progressive pigmentation of the skin since 1924. Pigmentation of the gums appeared at the same time. There have been numerous colds with chills and fever. Backache has been marked. There has been no pain in the chest and no night sweats, cough or hemoptysis. One month ago the patient had an attack of profuse sweating associated with delirium for one day. The sweating was largely localized to the face. Since this attack the patient has been nauseated and has had several attacks of vomiting. During the past four years the patient has lost 40 pounds. There are three children, aged 4 years, 2 years, and 11 months, living and well. There were two miscarriages.

Aside from marked pigmentation of the skin and the mucous membrane, *physical examination* revealed nothing of significance except a low blood pressure. Blood pressure: systolic, 90 and diastolic, 50; systolic, 92 and diastolic, 52; systolic, 80 and diastolic, 50. Blood sugar, 72, non-protein nitrogen, 27.3 and uric acid 41.3. Wassermann negative. Basal metabolism: −10 to −16. Temperature, pulse, respiration and urine normal. Blood count: red blood cells, 4,280,000; white blood cells, 5,500; polymorphonuclears, 39 per cent; eosinophiles, 3.6 per cent; lymphocytes, 53 per cent; and mononuclears, 4 per cent.

During stay in the hospital there were several attacks similar to the one a month before admission. The patient failed to respond to ephedrine, suprarenal gland and stimulants and died April 25, 1926.

*Clinical Diagnosis*. Addison's disease.

NECROPSY. Two hours postmortem. Limited to abdominal incision.

*Heart*. Weight 156 gm. There is an old mitral rheumatic endocarditis.

*Lungs.* Both lungs show puckered scars at the apex (healed tuberculosis) and small areas of bronchopneumonia.

*Spleen.* Weight 125 gm. Large in comparison with the size and weight of the other organs. Malpighian corpuscles large and hyperplastic on microscopic examination.

*Liver.* Weight 1040 gm. Appears normal.

*Pancreas.* Appears normal in gross. Section shows the islands of Langerhans more numerous and larger than usually seen.

*Gastro-Intestinal Tract.* Lymphoid follicles and Peyer's patches large.

*Kidneys.* Weight 153 gm. Normal.

*Adrenals.* No adrenal glands could be demonstrated on gross examination and no accessory adrenal tissue could be found around or in the kidneys, along the ureters or in any of the pelvic tissue. The tissue in the region where the adrenals are normally found was dissected out and preserved for microscopic study. Section of this tissue showed the structure of the adrenal gland with here and there small clumps of cortical or of medullary adrenal tissue. Throughout the glands there was extensive lymphocytic infiltration. The areas which contained adrenal parenchyma showed some of the parenchymal cells necrotic and invaded by mononuclear and polymorphonuclear leucocytes. No evidence of tuberculosis was found. Semilunar ganglia negative.

*Ovary.* Small simple cysts.

*Diagnoses.* Marked atrophy and destruction of adrenal tissue associated with hyperplastic lymphoid tissue; old rheumatic endocarditis; old healed bilateral pulmonary tuberculosis; bronchopneumonia; and hyperplastic islands of Langerhans.

## Discussion

Essential or idiopathic adrenal insufficiency with the Addisonian syndrome appears to occur more commonly in European countries than in America, if one may judge from the literature.

Wiesel [2] after a study of several cases of Addison's disease came to the conclusion that the primary lesion was in the chromaffin tissues. In most of his cases he noted the presence of hyperplastic lymphoid tissue with thymic and splenic enlargement.

Hedinger [3] in an analysis of fifteen cases of Addison's disease, fourteen of which showed tuberculosis of the adrenals, found a com-

mon occurrence of the picture of status thymico-lymphaticus. He agrees with Wiesel that the essential pathologic lesion in these cases is in the chromaffin tissue.

Bittorf [4] collected from the literature forty-seven cases of Addison's disease in which necropsy had shown absence of tuberculosis or tumor growth in the adrenals. To this series he added five cases of his own, three of which came to necropsy and showed the same condition. In a study of the adrenal in these cases, three showed involvement of the cortex only, and the remainder showed involvement of the whole organ. In all but one case there was present a hyperplastic condition of the lymphoid tissues. Both sexes were about equally involved, there being twenty-three males and nineteen females. The age range was 10 to 50 years in the male and 18 to 65 years in the female. In the majority of instances in which the sympathetic nerve ganglia were studied, these structures appeared normal. Bittorf's conclusion is that from the anatomic side the essential lesion is a disease of both adrenals and that the sympathetic nerve system is seldom involved. He also believes there is a relation between status thymico-lymphaticus and Addison's disease.

Marine, *et al.*,[5] from evidence obtained by removal of the adrenals, gonads and thyroid in the rabbit, conclude that removal of the thyroid hastens the involution of the thymus and removal of the adrenals or gonads not only retards this involution but brings about a condition where there is an actual regeneration of the thymus. Removal of both the gonads and adrenals produces this result more strikingly than the removal of the one or the other. They conclude that "the so-called lymphatic constitution which underlies or accompanies exophthalmic goiter, Addison's disease, and acromegaly also appears to be dependent on a partial suppression of certain functions of the inter-renal and sex glands."

Jaffe [6] obtained similar results as the above in rats following removal of the adrenals. He states: "It is now generally accepted that both in Addison's and in Grave's disease regeneration of the involuted thymus occurs, which may take place even in the presence of profound emaciation or chronic infection. We are of the belief that the large thymus which occurs in status lymphaticus, and the regeneration which occurs in Addison's and Grave's disease are brought about by the same disturbances of glandular inter-relations which bring about regeneration of the thymus in the experimental

animal after suprarenalectomy." He also states: "The mechanism involved in the regeneration of the thymus which follows suprarenalectomy is still not understood, and while thymic hyperplasia may be one manifestation of the generalized lymphoid hyperplasia that follows sublethal but sufficient suprarenal injury, nevertheless, the fact should not be lost sight of that thymic enlargement may represent a specific reaction of this organ to suprarenal injury."

From the above brief survey of the literature the two cases here recorded show several points of interest. They both belong to the "essential or idiopathic adrenal insufficiency," as classified by Bittorf. In both cases there was present a marked hyperplasia of the lymphoid apparatus, including the spleen in both cases and the thymus in the one case where examination was possible. Neither case showed any clinical or pathologic evidence of hypofunction of the gonads. In the case of the female, pregnancy and healthy children had ensued. In the case of the male, the testicles were functional and appeared normal in the gross and the microscopic examinations.

These cases emphasize the common occurrence of the pathologic picture of status thymico-lymphaticus in association with Addison's disease. That cases of status thymico-lymphaticus occur without the clinical syndrome of Addison's disease is common knowledge. The experimental evidence cited above gives proof of thymic regeneration and general lymphoid hyperplasia where sublethal suprarenal injury has been produced. It seems quite plausible, therefore, that the Addisonian syndrome is produced only in cases of extreme adrenal insufficiency and that status thymico-lymphaticus is but an indication of adrenal insufficiency, being present in both sublethal and lethal cases.

The etiologic factor or factors causing adrenal insufficiency, aside from destruction of the adrenal tissue by infections, such as tuberculosis, or by tumor growths within the organ, are not at present understood. A popular conception is that there is a disturbed balance between the secretions produced by the ductless glands. Whether this is the real factor remains to be proved. If, however, status lymphaticus may be taken as an indication of hypofunction of the adrenals, the greater frequency of the lymphatic constitution in children and young adults would suggest these cases have deficient adrenals at birth. The degree of deficiency would, in association

with the mode of life, determine the date at which clinical manifestations of adrenal insufficiency would appear. Gross and microscopic examination of the adrenals in many of these cases would be negative.

The fact that toxic necrosis of the adrenal parenchyma occurs in various infectious diseases has been clearly demonstrated by Graham[7] and others. In such cases there is evidence of regeneration of the parenchymal cells. It seems apparent that, because of the absence of clinical manifestations of adrenal insufficiency subsequent to recovery from these infections, the regeneration following necrosis is sufficient to permit of normal adrenal function. Although in the two cases here recorded there did not appear to be any etiologic connection with any infection, still it must be considered a possibility that during some infection there had occurred extensive destruction of the parenchyma of the adrenal with insufficient regeneration for continued proper adrenal function.

The hyperplastic condition of the lymphoid tissues may be explained in one of two ways, the first being that the adrenal under normal conditions exerts a suppressive or regulating effect upon the thymus and other lymphoid tissues. This view is expressed by Jaffe and Marine, *et al.* The second explanation is that, with the removal of the adrenal secretions, toxic products of metabolism accumulate and that cells of the lymphoid tissues are called out in large numbers in an attempt on the part of the body to detoxify such substances. The rôle that the lymphocytes play in various reparative processes in the body would make it seem that this second explanation of the lymphoid hyperplasia were the more plausible. Also the extensive lymphocytic infiltration of the damaged adrenals would suggest a reparative process. There appears to be no evidence of lawlessness of growth in the hyperplastic lymphoid tissue in these cases. There appears, however, to be a far greater demand for the lymphoid elements in adrenal insufficiency than in other disease processes. This explanation would also fall in accord with Warthin and others who have noted a lymphoid exhaustion in a large percentage of these cases.

## Conclusions

Two cases of essential adrenal insufficiency with the Addisonian syndrome are recorded.

The association of status lymphaticus and hypofunction of the adrenals is further emphasized.

An impression is gained that status lymphaticus is an expression of an attempt on the part of the body to correct a deranged metabolism brought about by a paucity of adrenal secretion.

The cases here recorded gave no clinical or pathologic evidence of gonad insufficiency.

I wish to express my appreciation to the medical service of the Wisconsin General Hospital and of the Student Infirmary for the privilege of incorporating in this article a brief summary of the clinical history of the cases herein reported.

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7. Graham, G. S. Toxic lesions of the adrenal gland and their repair. *J. Med. Res.*, 1916, xxxiv, 241.

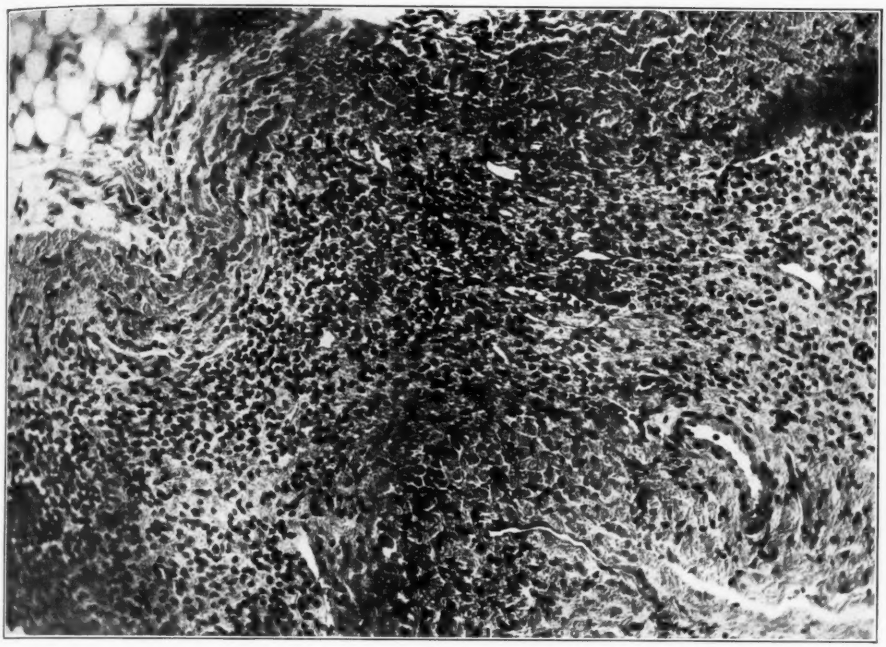
## DESCRIPTION OF PLATE

### Plate 55

Fig. 1. Adrenal from Case B, showing absence of adrenal tissue and marked lymphocytic infiltration of the organ. × 100.

Fig. 2. Adrenal from Case H, showing a few parenchymal cells of the cortex. Some of these cells are markedly hypertrophied. There is also considerable lymphocytic infiltration of the organ. × 250.

Fig. 3. Adrenal from Case B, showing a few of the parenchymal cells of the medulla still present. Note the extreme degree of lymphocytic infiltration. × 300.



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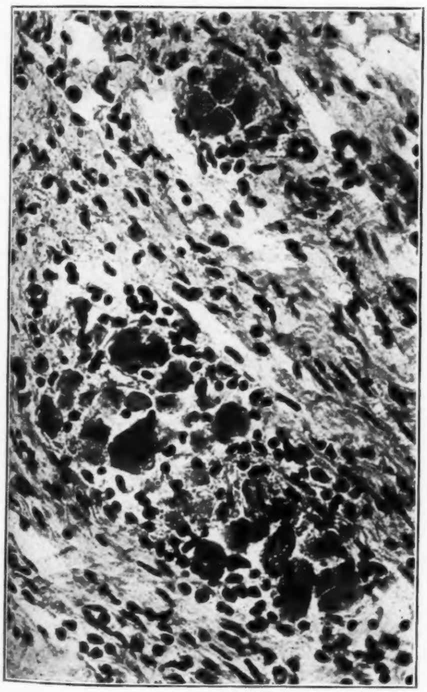

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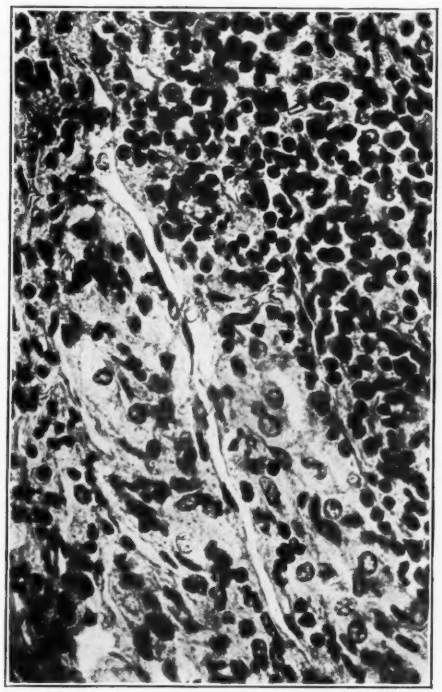

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Medlar

Essential Adrenal Insufficiency

# I. TRANSPLANTATION AND INDIVIDUALITY DIFFERENTIAL IN STRAINS OF INBRED RATS*

Leo Loeb and Helen Dean King

(*From the Department of Pathology, Washington University Medical School, St. Louis, Mo., and the Wistar Institute, Philadelphia, Pa.*)

In a preceding paper one of us has reported on the transplantation and individuality differential in inbred families of guinea-pigs which were obtained from the Department of Agriculture in Washington.[1] In this paper we wish to report on similar experiments in rats and while it is published subsequently to the corresponding paper on guinea-pigs, the experiments on inbred rats were begun at an earlier time than those on guinea-pigs. However, the results in the latter were so much more in accordance with expectations, that it was thought best to publish them first in order to contrast with them the results obtained in rats. The rats used had been inbred by Miss King in the Wistar Institute, Philadelphia,[2] and the experiments on both guinea-pigs and rats were carried out in the Department of Pathology and Comparative Pathology of Washington University Medical School.

Two strains of rats, originally derived from the same lot, were inbred through many successive brother and sister matings. Thus a strain "A" and a strain "B" of inbred rats were separated. However, there existed a difference in the mode of inbreeding in rats and guinea-pigs; in the case of the rats there was selective breeding, in each case the most vigorous individuals being used, while in the case of the guinea-pigs the breeding was not selective. Thus Miss King did not observe a deterioration in the vigor of the rats in the course of long continued inbreeding, while in the case of the guinea-pigs a definite, although on the whole moderate, deterioration has been established by Dr. Wright.

We carried out the following series of experiments. Series A: We transplanted tissues from rats of inbred strain A to other not directly related A rats. Series B: In a similar way we carried out corresponding transplantations within the B strain. In both the A

An accident has made it necessary to publish this paper first. The report on the transplantation and individuality differential in inbred guinea-pigs will soon follow.

* Received for publication December 13, 1926.

and B strains the rats used belonged to the 37th and 38th inbred generation (subseries I), or to the 46th and 47th generation (subseries II). In further experiments, instead of using not directly related rats we carried out the same transplantations between brothers in the A as well as in the B strain, and again earlier (40th and 41st generation) as well as later generations (46th and 47th generations) were used. Lastly as control experiments we transplanted tissues from A to B strain and from B strain to A strain, in the earlier as well as in the later generations.

We see thus that the inbreeding within the rat strains A and B has progressed much further than within the guinea-pig strains. In the case of the guinea-pigs we used, in the large majority of our experiments, animals belonging to generations varying between the 15th and 23rd, while the rats used were from generations ranging between the 37th and 47th. We should therefore have expected that the similarity between the individuality differentials of donor and host would be much closer in the latter than in the former; however, our experiments proved that the opposite condition holds true.

## Series A. Transplantations from Rats of Strain A to other Rats of Strain A

In these experiments the donor and host did not belong to the same litter; they were therefore not brothers or sisters, nor did the exchange of tissues take place between parents and children; however a more distant relationship existed in all cases. Donor and host belonged to different litters which represented the 37th or the 38th generations, although in some cases the litter of the donor belonged to one, and the litter of the host belonged to the other, of these generations.

*Subseries I*, in which rats of the 37th and 38th inbred generation were used. Twenty experiments were made in this series; time of removal of transplant from host and examination of transplant varied in different cases between 19 and 45 days, the large majority having been examined between the 24th and 31st day. In eight cases a homoio-reaction was obtained; in two further cases the reaction was only slightly less intense. In six cases a syngenesio-reaction was found and in four cases the result approached conditions characteristic of autotransplantation. In 50 per cent of the cases

we obtained therefore a marked reaction against the transplant on the part of the host.

The grades in the various experiments were as follows: 3.25; 2; 1; 2.5; 1.5; 5.75; 3.5; 3; 5.5; 5; 3.25; 3.5; 4.50; 1; 1; 4.75; 5.25; 4.25; 3.5; 3.75. The average grade was 3.4. We shall cite a number of abstracts of the results obtained.

1. *24 days.* Thyroid transplant shows large masses of acini, either with or without colloid. There is dense fibrous tissue in the center. Between the ring of acini and center and in periphery of transplant there is a very marked lymphocytic infiltration; lymph vessels are filled with lymphocytes. In the cartilage transplant there is much lymphocytic infiltration in the fat tissue and in the connective tissue around the cartilage. Some lymphocytes penetrate into perichondrium and into newly formed cartilage, destroying the margin of it. Where cartilage is necrotic, perichondrium produces new cartilage around it. Some connective tissue penetrates between necrotic and regenerating cartilage. Grade 3.25.

2. *24 days.* The central part of thyroid is composed of hyaline connective tissue; the periphery of transplant consists of masses of lymphocytes among which are a few compressed acini; almost all the acini have been destroyed and their place taken by lymphocytes. In cartilage transplant, the cartilage is on the whole well preserved; where parts are necrotic, regeneration and a new formation of cartilage has taken place. In the surrounding fat tissue and connective tissue there is much lymphocytic infiltration. In some places lymphocytes penetrate to perichondrium. Grade 2.

3. *22 days.* Thyroid transplant destroyed; only fibrous tissue with lymphocytes left. In cartilage transplant, definite lymphocytic infiltration in surrounding connective tissue. Perichondrium produces new cartilage. Grade 1.

4. *25 days.* In thyroid transplant a good ring of acini, closely adjoining each other; good colloid. Loose connective tissue and large vessels in center. No lymphocytes. Cartilage surrounded by fat tissue. Around necrotic areas some perichondrial regeneration of cartilage. In the fat tissue some collections of lymphocytes. Grade 5.75.

5. *29 days.* Many acini of thyroid transplant with good colloid; some are without colloid. A number of acini in ring still closely adjoining each other; well developed vessels penetrate through ring

into center of piece. The central parts of thyroid ring are intensely infiltrated by lymphocytes, so that the appearance of a lymph gland is produced. Also in the peripheral parts of the thyroid ring there are some collections of lymphocytes. Grade 3.5.

6. Thyroid transplant well preserved; acini with or without colloid. Fat and a small amount of connective tissue in center. Well preserved parathyroid; in the center of it are some masses of lymphocytes around vessels. Lymphocytic infiltration in one pole has destroyed a considerable amount of thyroid tissue; but on the whole the lymphocytic infiltration is not very pronounced. Ovary shows good germ epithelium cyst. Well preserved large follicles; also atretic large follicles and follicles in the stage of connective tissue abresia; some primordial follicles with preserved ova. Yellow interstitial tissue. Medullary ducts with preserved Fallopian tubes well preserved; occasionally a few polymorphonuclear leucocytes in lumen. In the connective tissue in and around ovary slight lymphocytic infiltration. In uterus unstriated muscle tissue preserved. Grade 4.50.

7. In another similar transplant there are in addition to above findings new corpora lutea with many capillaries formed in the transplanted ovary. Grade 4.75.

8. *45 days.* Uterus transplant shows glands markedly proliferating with high epithelium. Some dilated glands with flat epithelium. Certain gland cells secrete mucus. Fibrillar connective tissue around glands. A number of lymphocytes migrate through epithelium. In places a few polymorphonuclear leucocytes in the interstitial tissue. Connective tissue cells and some lymphocytes in mucosa around epithelium, the latter lymphocytes migrating into the epithelium. A great amount of unstriated muscle preserved. In center of transplant hyaline fibrous tissue. Fallopian tubes almost unchanged; epithelium forms papillae. In ovary a great quantity of so-called interstitial gland consisting of large fairly vacuolar cells, with yellow pigment. A few primordial follicles with eggs. Some degenerating eggs in other follicles. Large system of medullary canals and germ epithelium prominent. Some nests of cells without ova in cortex. Fat tissue with blood pigment. Around a vessel in ovary a collection of lymphocytes. Grade 5.25.

9. *31 days.* Thyroid. Acini preserved with colloid; also parathyroid preserved. Large blood vessels surrounded by lymph

vessels which are filled with lymphocytes. A great deal of lymphocytic infiltration, in places very dense. Lymphocytes seem to migrate from the tissue into the blood vessels rather than in the opposite direction. In transplanted uterus and Fallopian tube muscle tissue preserved. Epithelium surrounded by dense fibrous tissue; both structures noticeably infiltrated with lymphocytes. In the walls of the tubes also some lymphocytic infiltration. In the surrounding transplanted fat tissue especially large masses of lymphocytes. One ovary well preserved. Germ epithelium, tunica albuginea and yellow interstitial tissue preserved; also medullary ducts. In tunica albuginea small apparent follicles consisting of granulosa cells without ova. Degenerated corpus luteum with very vacuolar and a few preserved lutein cells. On the whole, little lymphocytic infiltration in this ovary. The other ovary is well preserved also. Small follicles with ova; large follicles with large cavities and good ova. Germ layer partly infiltrated with lymphocytes; many mitoses in germ epithelium. In general slight, but in places more marked, lymphocytic infiltration in this ovary. Grade 4.25.

*Subseries II.* Inbred rats of the 46th and 47th generation were used in four experiments. The pieces were in all cases removed for examination thirty days after transplantation. Grades 1; 1; 1.25; 1.25. The results were those characteristic of typical homoiotransplantation.

We shall cite two abstracts. 1. *30 days.* From rat of third litter of 46th generation to first litter of 47th generation. In thyroid transplant only fibrous tissue found. Around cartilage increase of fibrous tissue and a discontinuous, but distinct, mantle of lymphocytes. Connective tissue and lymphocytes invade cartilage; instead of areolar and fat tissue we find fibrous tissue. Muscle is necrotic. Bone marrow is replaced by connective tissue with lymphocytes. Around bone, giant cells and lymphocytic infiltration. Vessels, connective tissue and lymphocytes penetrate into bone. Grade 1. 2. *30 days.* From first litter of 47th generation to second litter of 46th generation. Thyroid transplant shows only fibrous tissue. Around necrotic cartilage large plate of perichondrial cartilage. Much fibrous tissue and many coalesced fat cells. In areolar tissue much newly formed connective tissue with collections of lymphocytes. Around cartilage, moderate and discontinuous, but distinct, mantle of lymphocytes.

Comment. The average grade of the earlier experiments with the 37th and 38th generation is 3.4; that of the later experiments with the 46th and 47th generation is 1.12. Of course the number of experiments in the latter subseries is only four. The average grade of results in Series A (both subseries combined) is 3. Transplants of thyroid, parathyroid, cartilage, uterus, Fallopian tubes and ovaries were used as a test in grading the severity of the reaction on the part of the host. The behavior of these organs does not differ in any essential respect from other previous findings which one of us described in earlier papers. Lymphocytes and connective tissue of the host invade and damage the tissues; in addition a direct injurious effect of the homoio-toxins may be observed in the case of sensitive tissues. The ovary is usually invaded by lymphocytes to a much less extent than thyroid or parathyroid. Corpora lutea may develop through rupture of the follicles; in addition atresia may take place in follicles which do not have a chance to rupture. In general there is again a parallelism in the behavior of all the tissues from one animal which are transplanted into another. We shall compare the results obtained in this series with those obtained in the case of non-inbred rats, after we have reported on all the experiments with inbred rats.

## Series B. Transplantations from Rats of Strain B to other Rats of Strain B, Belonging to Different Litter

*Subseries I*, in which rats of the 37th and 38th generation were used. There were twenty-two experiments. The pieces were removed at periods varying between 14 and 37 days following transplantation. In two cases the results approached an auto-reaction; in seven cases a syngenesio-reaction was obtained; in the remaining thirteen cases the result corresponded to a homoio-reaction or approached this condition. The grades in the individual experiments were as follows: 2.75; 1.25; 1; probably 1–2; 4; 1; 2; 4.25; 4; 2; 5; 5; 1.5; 1; 1; 5; 1.25; 5.50; 5; 4.5; 5.50. Average grade 3.0 (or 3.1). We shall cite some examples which illustrate especially the behavior of organs other than thyroid and cartilage.

1. *36 days*. Inbred rats of the 36th generation. Thyroid not preserved; only fibrous tissue with lymphocytes. Ovary contains medullary ducts which are much infiltrated with lymphocytes; also

a germ epithelium cyst which is also much infiltrated. There are good large follicles, the theca interna of which shows some infiltration; but at some distance from the follicles there is a larger number of lymphocytes. Small to medium-sized good follicles and primordial follicles with ova; also atretic follicles. In connective tissue much infiltration with lymphocytes and lymph vessels are studded with these cells. Fallopian tubes and uterus also very much infiltrated. Grade 1.5.

2. *30 days*. Inbred rats of the 36th generation. Thyroid transplant destroyed; only fibrous tissue with small masses of lymphocytes. Uterus and ovary have been destroyed by fibrous tissue and some lymphocytes around vessels. Grade 1.

3. *25 days*. Inbred rats of the 37th generation. Instead of thyroid only fibrous tissue with some foreign bodies and foreign body giant cells visible and a very considerable lymphocytic infiltration in the fibrous tissue. Uterus transplant is replaced by fibrous tissue, with slight lymphocytic infiltration. Some interstitial tissue is the only remnant of ovary. Grade 1.

4. *37 days*. Thirty-seventh generation. Thyroid. A ring of acini with firm colloid is surrounded by a fibrous capsule; acinus cells of medium height. Loose connective and fat tissue and vessels in center, also some fibrous tissue and nests of squamous epithelium. Some large vessels penetrate through thyroid ring into center. Lymph vessels in center studded with lymphocytes; at poles of transplant and around the large vessels are masses of lymphocytes. In center of thyroid are many scattered cells of this type. Except for the presence of the lymphocytes the thyroid resembles an autotransplant. The parathyroid is well preserved. In its periphery there are masses of lymphocytes around a vessel which penetrates somewhat into the parathyroid proper. Uteri and Fallopian tubes like autotransplants; epithelium, glands, connective tissue and muscle tissue are normal, but there are here and there small collections of lymphocytes. The ovarian transplant consists of a germ epithelium cyst, large good follicles with well developed granulosa containing many mitoses; primordial follicles with ova; also atretic follicles and interstitial tissue in various stages of development. In the fibrous tissue around transplant some masses of lymphocytes around vessels. Grade 5.

5. *32 days*. Thirty-sixth generation. Liver transplant. A great

part of the transplanted tissue is necrotic. In the peripheral connective tissue are collections of bile ducts which are surrounded by fibrous or dense fibrillar connective tissue. There are areas of liver cells around bile ducts and around vessels. Some of these cells have two nuclei and some become vacuolar. Lymphocytes are found lying around liver cells and some may penetrate into the latter. In peripheral fibrous tissue are lymph vessels with lymphocytes and a certain amount of lymphocytic infiltration. Yellow bile pigment in the connective tissue. In places many eosinophiles and lymphocytes around transplant. A number of the lymphocytes seem to form plasma cells. Around the necrotic liver tissue some foreign body giant cells are produced. Grade 5.50.

6. *32 days.* Thirty-sixth generation of inbred rats. Kidney transplant. A great part necrotic; but quite a number of straight tubules in periphery of transplant and a few glomeruli preserved. There is a fair amount of lymphocytic infiltration; lymph vessels are filled with lymphocytes and these cells penetrate also into some living tubule cells. Also in periphery, fat and connective tissue are infiltrated with lymphocytes. Grade 5.

7. *32 days.* Thirty-sixth generation of inbred rats. Spleen transplant. Trabeculae with blood pigment cells; polygonal vacuolar, phagocytic cells, some of which take up blood pigment. Certain of these cells have much enlarged nuclei, especially those which penetrate into the necrotic material and take the latter up. Also megakaryocytes are found. In periphery of transplant some masses of lymphocytes. Grade 4.5.

COMMENT. The average grade in subseries I of Series B is 3 or 3.1 which is slightly below the average grade of the subseries I of Series A. As usual in various experiments the grades differ very much, the variations ranging between 5.6 and 1. Of special interest in this subseries is the behavior of various organs other than thyroid and cartilage. We notice a parallelism in the behavior of different tissues transplanted from the same donor to the same host. When thyroid has been destroyed, great parts of ovaries and uterus may be preserved, but they show very marked lymphocytic infiltration which may extend even to the follicles; in other cases, in which the homoioreaction is still more severe, not only thyroid is replaced by fibrous tissue, but also uterus and ovaries; the interstitial tissue of the ovary is one of the last tissues to disappear. On the other hand, when

thyroid and parathyroid are well preserved and show only a small amount of lymphocytic infiltration, uterus and ovary also are well preserved and are only slightly infiltrated. As one of us found previously in favorable cases, liver cells as well as bile ducts may be preserved; but also here lymphocytes begin to infiltrate the transplant and they may invade liver cells. Foreign body giant cells may form around the necrotic liver tissue. In favorable kidney transplants especially the straight tubules and glomeruli may be preserved, and in the spleen, trabeculae, megakaryocytes and perhaps some lymphocytic masses are found. Cells which invade and replace through phagocytosis the central necrotic part of the transplant may assume the character of vacuolar polygonal cells often possessing very large nuclei.

*Subseries II*, in which rats of strain 3 of the 46th and 47th generation were used. Six experiments constitute this subseries. Removal of transplanted pieces took place 25 and 35 days after transplantation.

The following grades were obtained: 1. From first litter 47th generation to third litter 46th generation, 35 days. Grade 1.

2. From first litter 47th generation to third litter 46th generation, 35 days. Thyroid with auto-structure, but much lymphocytic infiltration; lymph vessels studded with lymphocytes. In center and also in periphery a great deal of lymphocytic infiltration. Lymphocytes penetrate between and also in acini and destroy them. Strands of squamous epithelium in the central fibrous tissue also infiltrated with lymphocytes. A large number of acini still preserved and closely adjoining each other; some acini without colloid. Fibrous tissue surrounds cartilage and replaces areolar and fat tissue; lymph vessels studded with lymphocytes. There is also diffuse lymphocytic infiltration. Bone preserved, but around it is marked lymphocytic infiltration. Lymphocytes mingle with osteoblasts. Grade 3.5.

3. From first litter 47th generation to third litter 46th generation, 25 days. Grade 1.5.

4. From first litter 47th generation to third litter 46th generation, 25 days. In thyroid very intense lymphocytic infiltration. Lymph vessels studded with lymphocytes. Masses of these cells penetrate between and into acini and destroy them. Colloid in acini disappears partly through the activity of phagocytes. Many empty acini which are then compressed and invaded by lymphocytes and

probably by connective tissue cells as well. Fibrous tissue surrounding acini increased and much increase in central connective tissue. A plate of perichondrial cartilage lying at side of necrotic cartilage. In areolar and fat tissue around cartilage much fibrosis and many confluent fat cells; around blood vessels collections of lymphocytes. Bone surrounded by foreign body giant cells; bone marrow is not preserved. Apparently osteoblasts penetrate into necrotic cartilage, destroy it and form bone. Grade 2.75.

5. From first litter of 47th generation to second litter of 46th generation, 35 days. Grade 3.

6. From first litter of 47th generation to second litter of 46th generation, 35 days. In center of thyroid areolar tissue and a little fibrous tissue. Good ellipsoid of acini. In center epithelial pearls. Some lymph vessels filled with lymphocytes. At one pole of transplant considerable lymphocytic infiltration around vessels. Well preserved parathyroid. Large parts of cartilage also well preserved, surrounded by areolar and fat tissue with little lymphocytic infiltration and small amounts of connective tissue; but some newly formed fibrous tissue is found. Around necrotic cartilage plate of perichondrial cartilage. Tissue mostly free from lymphocytes and connective tissue.

Comment. The average grade of this subseries is 2.8, which is slightly less than the grade of subseries I. In strain A, the thyroid of second subseries is also below that of first subseries, but the difference between the grades of the two subseries is greater in Series A than in Series B. The average grade of Series B is 2.92, a figure slightly below that of Series A. The correspondence in the behavior of thyroid, parathyroid, cartilage and bone marrow in the different cases is very evident in these experiments; only when the reaction against thyroid and cartilage is slight are bone marrow partly preserved and megakaryocytes found. Grade 5.

## Series C. Transplantation from Brother to Brother in Inbred Rats

*Subseries I.* Rats of strain B of the 40th and 41st generation were used; eighteen experiments; pieces were taken out 25 to 40 days after transplantation. Grades were as follows: 1. *25 days.* Thyroid, parathyroid and cartilage. Grade 6. 2. *30 days.* Thyroid, cartilage,

uterus and ovaries. Grade 6. 3. *35 days.* Cartilage and uterus. Grade 6. In the uterus transplant there were good papillae and mitoses in the unstriated muscle tissue; myxoid connective tissue separates epithelium from fibrous tissue. In one place some lymphocytic infiltration beneath epithelium. The muscle is hypertrophic. In this case transplantation had taken place into a sister which became pregnant subsequent to the transplantation; this explains probably the condition found in the uterus. 4. *40 days.* Grade 4.5. Distinct lymphocytic infiltration. These four animals belong to the same litter. 5. *40 days.* Uterus and ovaries. Grade 6. 6. *29 days.* Grade 2.25. Thyroids with much fibrous tissue and large blood vessels in center; few acini are left. Each of the preserved acini is surrounded by fibrous tissue. Definite lymphocytic infiltration. Around cartilage marked lymphocytic infiltration. Also in fat tissue lymphocytic infiltration. Some regenerated perichondrial cartilage. 7. *35 days.* Grade 5.25. Structure corresponds to autotransplant. Slight lymphocytic infiltration around thyroid and parathyroid. These three animals belong to the same litter.

8. *40 days.* Grade 5.25. Thyroid and cartilage resemble autotransplants, except that at one pole of former there is marked lymphocytic infiltration; lymphocytes destroy here part of thyroid. 9. *30 days.* Grade 5.5. Thyroid and cartilage resemble autotransplants except for slight collections of lymphocytes. Animals in last two experiments belong to the same litter.

10. *35 days.* Grade 4.25. Thyroid and cartilage show structure of autotransplants, but now intense lymphocytic infiltration destroys part of thyroid and cartilage transplant contains a number of lymphocytes. 11. *25 days.* Grade 4.25. Cartilage and uterus are on the whole well preserved, but show in places marked lymphocytic infiltration. In uterus much diffuse lymphocytic infiltration where vessels enter. At certain points marked infiltration of mucosa. 12. *35 days.* Grade 5.5. Thyroid, parathyroid and cartilage like autotransplants, but a slight lymphocytic infiltration in places. Animals in last three cases belong to the same litter.

13. *25 days.* Grade 4.75. Thyroid consisting of good ring of acini with much colloid; centre with considerable dense connective tissue. Extensive lymphocytic infiltration invading center of thyroid and part of parathyroid. Lymph vessels filled with lymphocytes. Cartilage surrounded by fat tissue. Around necrotic cartilage peri-

chondrial cartilage proliferation. Trace of lymphocytes. 14. *25 days.* Thyroid and cartilage. Grade 5. 15. *25 days.* Thyroid, parathyroid and cartilage. Grade 6. 16. *41 days.* Thyroid and cartilage. Grade 6. 17. *41 days.* Thyroid and cartilage. Grade 6. 18. *41 days.* Thyroid and cartilage. Grade 5.25. These six animals all belonged to the same litter.

COMMENT. The grades are 6 in seven experiments; 5.5 in two experiments; 5.25 in three experiments; 5, 4.75 and 4.5 each in one experiment; 4.25 in two experiments; 2.25 in one experiment. The average grade is 5.21. The rats used in these eighteen experiments belonged to five different litters; the results within the same litter differ according to the character of donor and host. The average strength of reaction is here much less marked than in the case of transplantation from one litter to the other.

*Subseries II.* Transplantations from brother to brother in inbred rats of strain A of the 42nd generation. Six experiments were made. Pieces were removed for examination at periods varying between 25 and 40 days after transplantation. The following grades and conditions were found: 1. *25 days.* Grade 4. Thyroid and parathyroid on the whole well preserved, but marked lymphocytic infiltration especially at inner edge of thyroid ring. Lymphocytic masses destroy some tissue. In places connective tissue separates acini; but lymphocytes act independently of connective tissue. Cartilage on the whole well preserved. Some necrosis. In connective tissue around cartilage distinct lymphocytic infiltration.

2. *40 days.* Grade 4.5. Condition of thyroid, parathyroid and cartilage similar to that in former experiments, but somewhat less lymphocytic infiltration; only slight infiltration between acini and only a few acini destroyed by lymphocytes. On the whole, well preserved cartilage. Some perichondrial proliferation in various places. Around perichondrium lymphocytic infiltration. Capillaries penetrate into necrotic part of cartilage. Where cartilage is thicker, it is necrotic *in toto.*

3. *25 days.* Grade 1.5. Thyroid destroyed. Cartilage surrounded by fat and fibrous tissue. Lymphocytic infiltration of variable intensity. Some slight perichondrial proliferation. Connective tissue buds growing into necrotic cartilage. Animals from the same litter served for these three experiments.

4. *25 days.* Grade 2.5. Thyroid infected. Cartilage transplant

shows much connective tissue reaction and some lymphocytic infiltration. 5. *32 days*. Grade 6. Thyroid, parathyroid and cartilage. The animals in these three experiments belonged to the same litter. 6. *25 days*. Grade 2.5. No thyroid found. In fat tissue around cartilage, epithelial and giant cell reaction; confluent fat cells. Lymphocytic infiltration. Perichondrial proliferation and nodules of newly formed cartilage.

COMMENT. The average grade of this subseries is 3.5, which is a distinctly lower figure than that of the preceding subseries. We must of course consider that the number of experiments here is relatively small, so that the same importance cannot be attached to this low average grade as to the higher one in the preceding subseries. Of interest are the observations which confirm previous ones, demonstrating that the lymphocytic infiltration of transplants may be independent of proliferation of connective tissue and that blood vessels penetrate only into necrotic parts of this tissue. Again the identity of the reaction in transplantations in the same litter may show distinct differences in different experiments. The average grade of subseries I and II combined is 4.78.

*Subseries III and IV*. In these subseries, transplants were made from brother to brother of the 46th and 47th generations of inbred A and B rats. These experiments correspond therefore to subseries II in both Series A and Series B in which exchange of tissues in inbred rats occurred in animals belonging to different litters. In subseries II of Series A and B we found the average grade distinctly lower than in the corresponding subseries I in which a somewhat earlier generation of inbred rats were used. In a similar way we find in the case of brother to brother transplantations with animals of the 46th and 47th generations a distinctly lower grade than in subseries I and II of Series C in which animals of the 40th to 42nd generations were used for transplantation.

*Subseries III*. Brother to brother transplantations in strain A of inbred rats of the 46th and 47th generations. 1. *45 days*. Grade 3.50. Thyroid, parathyroid and cartilage. Auto-structure. Intense lymphocytic infiltration has destroyed great parts of thyroid. Lymphocytes penetrate parathyroid also in direction from periphery toward center. Cartilage transplant in places shows some marked infiltration and some increase in connective tissue; some confluent fat cells.

2. *45 days.* Grade 1.5. Thyroid and cartilage. 3. *40 days.* Grade 1.5. No thyroid remains. Cartilage preserved surrounded by fibrous tissue; moderate lymphocytic infiltration. Myxoid connective tissue and a few lymphocytes replace bone marrow. Regenerated perichondrial cartilage cells are again degenerating.

4. *40 days.* Grade 3.25. Thyroid and cartilage. Great parts of thyroid and parathyroid destroyed, but areas preserved in which acini are close together. Intense lymphocytic infiltration. In cartilage transplant fibrous tissue has replaced considerable amounts of fat tissue. Imperfect mantle of lymphocytes around cartilage; in fat tissue, small collections of lymphocytes. Some muscle fibers with chains of nuclei; lymphocytes between muscle fibers.

5. *45 days.* Grade 1.25. Thyroid and cartilage. 6. *45 days.* Grade 1.25. Thyroid and cartilage.

Comment. The average grade of this subseries is 2.04 which is considerably lower than the average grade of subseries I and II. Individual grades: 3.50; 1.50; 1.50; 3.25; 1.25; 1.25. Of interest again is the good correspondence in the conditions of different tissues or organs transplanted into the same host. Thus when the grade is low in the case of thyroid or parathyroid, bone marrow is replaced by myxoid connective tissue and lymphocytes. On the other hand, if a syngenesio-reaction is obtained with thyroid or cartilage transplants, muscle fibers with nuclear chains may be found as late as forty days after transplantation, with a grade of 3.25.

*Subseries IV.* This subseries corresponds to subseries III except that rats of strain B were used instead of strain A, the animals belonging to the 46th and 47th inbred generation. This subseries includes five experiments in which the transplantation was from brother to brother and two further experiments in which the transplantation was from mother to child. The grades were as follows: 1. *22 days.* Grade 4. Thyroid, parathyroid and cartilage. Autostructure, but quite marked lymphocytic infiltration which has destroyed part of thyroid and parathyroid. Some slight increase in connective tissue in areolar and fat tissue around cartilage.

2. *35 days.* Grade 1.5. Thyroid, cartilage, bone and bone marrow. The latter has been replaced by myxoid connective tissue with some lymphocytes.

3. *35 days.* Grade 1.5. Thyroid and cartilage. Similar to experiment 2. No thyroid preserved; fibrous tissue around cartilage; some fat tissue preserved; some coalesced fat cells.

4. *40 days*. Grade 5.75. Thyroid, cartilage and bone marrow. Thyroid and cartilage well preserved with surrounding fat tissue; very slight collection of lymphocytes in thyroid. Bone marrow partly well preserved with megakaryocytes.

5. *40 days*. Grade 3.75. Thyroid, parathyroid and cartilage. Thyroid with auto-structure. Intense lymphocytic infiltration in thyroid and parathyroid; only remnants of these tissues remain. Strands of lymphocytes penetrate parathyroid and collect in center. Some good areas of thyroid left, where acini are close together and contain colloid; but lymphocytes penetrate also these parts entering between and into acini. Well preserved cartilage, surrounded by fat tissue in which fibrous tissue is increased. Many small strands of lymphocytes in areolar and fat tissue.

6. *25 days*. Grade 1.5. From mother of 46th to child of 47th generation. Thyroid and cartilage.

7. *25 days*. Grade 4. (The same litters as 6). Thyroid, parathyroid and cartilage. Auto-structure of thyroid; but increased connective tissue and lymphocytes; many acini destroyed. Also in fat tissue around cartilage some increase in connective tissue and marked lymphocytic infiltration.

COMMENT. The individual grades in this subseries are 4; 1.5; 1; 5.75; 3.75; 1.5; 4. The average grade is 3.1. As observed before, the grade is lower in the later than in the earlier generation. Of interest again is the correspondence in the behavior of various organs transplanted into the same host. When thyroid, parathyroid and cartilage transplants are well preserved, bone marrow is likewise preserved, while in cases in which the reaction against these other tissues is severe the bone marrow is replaced by connective tissue. The average grade of subseries III and IV is 2.63 as compared with the average grade 4.78 of subseries I and II in which the earlier generation of rats was used. The average grade of all the brother to brother transplantations is 4.03.

### SERIES D. CROSS-TRANSPLANTATIONS FROM STRAIN A TO STRAIN B AND FROM STRAIN B TO STRAIN A

The proper control experiments for transplantation within the inbred strains would be transplantations from animals belonging to strain A to animals belonging to strain B and *vice versa;* such trans-

plantations would give an indication as to the changes in the individuality differentials which the long continued inbreeding through brother to sister matings has produced. We therefore carried out a series of transplantations of this nature. In both of these series we used animals of the 37th and 38th generation (subseries I) as well as from the 46th and 47th generation (subseries II).

I. *Transplantation from strain A to strain B. Subseries I.* Thirty-seventh and 38th generation. Fourteen cases comprise this subseries. One case in which the piece was removed as early as six days after transplantation cannot be graded, although we can state that even at this early period there was a definite reaction against the transplant on the part of the host. In the other cases the time of removal of the pieces varied between 19 and 45 days. In the majority of cases thyroid (sometimes with parathyroid) and cartilage were used for transplantation; in other cases ovary and uterus with or without thyroid. The grades were as follows: *24 days*, 1; *24 days*, 1; *26 days*, 2 (?); *25 days*, 5; *26 days*, 4 (?); *29 days*, 5.50; *23 days*, 2; *19 days*, 3; *30 days*, 2.50; *45 days*, 1; *36 days*, 2.5; *24 days*, 4; *31 days*, 1. Average grade 2.65.

The following example may be cited: Strain A to strain B, 37th generation, 36 days. Thyroid has been destroyed; only fibrous tissue is found. In the transplanted ovary a germ epithelium cyst is found including papillary structures resembling the fimbria. Good follicles, medullary ducts and interstitial tissue, also some unstriated muscle tissue seen. We find here a very distinct lymphocytic infiltration in the ovary proper as well as in the surrounding tissue; some infiltration is seen also in the epithelium of the Fallopian tube and there is a considerable amount in the unstriated muscle. The lymph vessels are filled with lymphocytes. Grade 2.5.

Comment. The average grade of the reactions in this subseries is very similar to that found previously by one of us in the case of homoiotransplantation in non-inbred rats, where the average grade varied between 2.5 and 2.7. We find again a variation in the intensity of the reaction in the individual cases. The example which we cite brings out very well that the ovaries and Fallopian tubes are more resistant to the action of the homoio-toxins than the thyroid; but notwithstanding this absolute difference there is a correspondence between the reaction, inasmuch as in the ovary as well as in the Fallopian tube the lymphocytic reaction is quite marked in this case,

although as a rule lymphocytes are rather small in number in the ovarian transplants.

*Subseries II.* Forty-sixth and 47th generation. Eight cases. In all except in one case, in which the removal of the piece took place after 17 days, the pieces were removed at a period varying between 20 and 30 days. The grades of only seven cases are given, as in one case the grade was uncertain: *22 days*, 4.25; *30 days*, 3; *30 days*, 1.50; *30 days*, 3; *25 days*, 2.75; *20 days*, 1; *20 days*, 2.25. Two examples may be cited in which in addition to thyroid (with or without parathyroid) and cartilage, bone and bone marrow or striated muscle were transplanted.

1. *25 days.* Forty-sixth generation. Thyroid transplant with a large amount of fibrous tissue in center; much lymphocytic infiltration. A great part of the peripheral ring of acini is destroyed; the remaining acini lose their colloid, they collapse and lymphocytes enter and destroy them. Parathyroid also much infiltrated with lymphocytes. Lymph vessels studded with lymphocytes. Cartilage is on the whole preserved. Regeneration of the perichondrium leads to the formation of a cartilage plate and regenerated perichondrial cartilage penetrates also into necrotic cartilage. In the fat tissue around cartilage much increase in connective tissue and much lymphocytic infiltration in places. At one end of cartilage there are striated muscle fibers with nuclear chains, and some lymphocytic infiltration between them; but as usual there is less lymphocytic infiltration here than thyroid. Grade 2.75.

2. *20 days.* Forty-sixth generation. The thyroid transplant consists now mainly of hyaline fibrous tissue. In the periphery of this fibrous tissue there are a number of acini, mostly compressed and without colloid, a few still containing colloid. Fibroblasts migrate around the acini and form fibrous bands; lymphocytes penetrate between and into the collapsed acini and destroy them. Some lymph vessels in the fibrous tissue filled with lymphocytes. Around the cartilage transplant there is much fibrous tissue; also lymphocytic infiltration is seen. Into the thick necrotic cartilage, connective tissue with lymphocytes is penetrating from the side. Areolar and fat tissue is partly replaced by fibrous tissue. Instead of bone marrow we find fibrillar or myxoid connective tissue with some lymphocytes. Few osteoblasts are left; only peripheral bone is preserved; the center is necrotic. On the whole, there is here not much

lymphocytic infiltration. Where new formation of connective tissue takes place around the cartilage, hemorrhage is often found.

COMMENT. The average grade is in this subseries about the same as in the first subseries; there is therefore no definite difference between the earlier and later generations; such as we find in the transplantation within the inbred strains A and B. Again the result agrees with that obtained in ordinary homoiotransplantation. In the first case which we cite, it is interesting to note that with reactions which stand at the border-line between syngenesio- and homoio-reaction, striated muscle tissue can show certain regenerative phenomena, although lymphocytes invade the transplanted muscle. In the second case we find, with a typical homoio-reaction, myxoid fibrillar bone marrow, a condition which confirms our previous findings.

II. *Transplantation from strain B to strain A. Subseries I.* Thirty-seventh and 38th generation. Sixteen experiments were carried out; the time of examination varied between 19 and 50 days. Thyroid, parathyroid, cartilage, ovary and uterus were the principal organs or tissues used for transplantation. The grades in the different experiments are as follows: *19 days*, 1.75; *19 days*, 1; *23 days*, 2.75; *28 days*, 2; *19 days*, 1.5; *28 days*, 4; *25 days*, 3 (?); *35 days*, 1.5; *36 days*, 2; *25 days*, 3; *23 days*, 3; *23 days*, 3.5; *37 days*, 5; *50 days*, 5; *50 days*, 2; *39 days*, 1. The average grade is 2.6.

Some examples which show special points of interest may be cited.

1. *25 days.* Thyroid transplant consists mainly of fibrous tissue in which are situated compressed acini without colloid. The fibrous tissue surrounds bundles of these compressed acini. Lymphocytes collect around the collapsed acini, penetrate into them and replace them. Lymph vessels are filled with lymphocytes. The transplanted ovary consists of a germ epithelium cyst, in part of which the germ epithelium has been lost. In such places connective tissue proliferates into the cyst cavity. Small follicles are preserved, at least their granulosa is present, but the eggs have disappeared in some cases; there is lymphocytic infiltration around these follicles as well as in the interstitial tissue. Fallopian tubes, either with or without epithelium; there is lymphocytic infiltration. Much fibrous tissue in these transplants. Grade 3.

2. *36 days.* Thyroid has been destroyed; cartilage is surrounded by lymphocytic masses. The ovarian transplant again consists of a germ epithelium cyst, the epithelial lining of which has not been

completely preserved, owing to desquamation. There are some follicles with granulosa epithelium and some atretic follicles. Much lymphocytic infiltration. Grade 2.

3. *25 days*. This specimen shows conditions similar to specimen 1. The thyroid is compressed through much development of fibrous tissue. Acini are without colloid and are much infiltrated with lymphocytes, the lymph vessels being studded with these cells. In other places connective tissue cells grow around compressed acini and there is little lymphocytic infiltration; but in general the lymphocytes destroy the tissue over considerable areas. Ovaries show small and large good follicles, but again in some small follicles the ova have disappeared. The granulosa cells proliferate by mitoses. There is a germ epithelium cyst; neither in granulosa nor in the interior is there much lymphocytic infiltration. In uterus unstriated muscle tissue is preserved. There is some but not a dense lymphocytic infiltration; however, the lymph vessels in the uterus are studded with lymphocytes and the connective tissue of the transplant is much infiltrated. Grade 3.

4. *37 days*. Thyroid well preserved; large acini with high epithelium and good colloid. Large vessels in center; around the vessels lymphocytic masses and also around center some collections of lymphocytes. The structure is that of autotransplant. Parathyroid also well preserved. In uterine epithelium, mitoses. The mucosa is cellular and fibrillar and well formed muscle layer in uterus. Some fibrous tissue around uterus; only slight lymphocytic infiltration. Ovary with germ epithelium cyst; small follicles and atretic large follicles; some small isolated lymphocytic masses. Grade 5. In a fifth specimen with grade 2 examined after 50 days there is in the ovary a large follicle without good ovum, a germ epithelium cyst, and interstitial tissue. In the connective tissue of the transplant there is a very marked lymphocytic infiltration, while the uterus transplant has been invaded, destroyed and replaced by dense masses of lymphocytes.

COMMENT. The grade in this subseries is about the same as the grade with the reciprocal subseries strain A to strain B, 2.65 in the latter and 2.6 in the former. Where there is a marked reaction in the case of the thyroid, we find a correspondingly marked reaction in the case of the ovary and tube, although relatively the latter transplants are better preserved. In the ovaries the follicles develop

only incompletely and ova may be lost. There is definite lymphocytic infiltration in the ovary. Of interest is the fact that when the epithelial lining of the germ epithelium cyst has been lost, the connective tissue grows into the cyst. In these transplants also the epithelium exerts a restraining influence on the underlying connective tissue. The second case cited shows likewise a better preservation of the ovary than of the thyroid; but the ovary is not completely preserved as it would have been with a more favorable character of the individuality differentials and there is much lymphocytic infiltration. It is somewhat better preserved and less infiltrated with lymphocytes in the third case, in which the thyroid is similar to the first case, but in the surrounding fibrous tissue there is also in this instance marked lymphocytic infiltration. In the fourth case, we find thyroid, parathyroid and uterus much better preserved; the ovary, however, shows about the same structure as in the first, second and third cases, but the lymphocytic infiltration is here distinctly less. The ovary is therefore not as fine a reagent for differences in the individuality differentials as is the thyroid, but the activity of the lymphocytes gives also in the ovary, to a certain extent at least, a measure of the similarity or dissimilarity of the individuality differentials. In the fifth case, however, the injury of the ovarian tissue and especially of the uterus has progressed much further.

*Subseries II.* This subseries consists of only three experiments; the rats belonged to the 46th and 47th generation. Thyroid, parathyroid and cartilage were used for transplantation. The grades were as follows: *30 days*, 2; *30 days*, 1.25; *22 days*, 3. Average grade 2.1.

In the first experiment the thyroid is so densely infiltrated with lymphocytes that it resembles a lymph gland; parathyroid tissue alone remains. Cartilage is well preserved. A great amount of fibrous tissue replaces the areolar and fat tissue, but part is left. Very dense mantle of lymphocytes around cartilage and in fat tissue; lymph vessels filled with lymphocytes. Bone marrow is replaced by fibrillar connective tissue with lymphocytes. Grade 2.

Comment. The second subseries does not differ in any essential respect from the other subseries in this group of experiments. On account of the small number of experiments, not much importance can be attached to a slight lowering of the grade (2.1 as compared to

2.6 in the first subseries). The example cited shows well the correspondence in the reaction towards thyroid and cartilage; in both transplants the lymphocytic infiltration is very pronounced. As to the whole group in series D, the total average grade of transplantation from strain A to strain B is 2.61, while the total average of transplantation from strain B to strain A is 2.53. These two figures are about the same. The total grade of both kinds of transplantations, namely from strain A to B and B to A is 2.57 which corresponds very closely to the grade of ordinary homoiotransplantation among non-inbred white rats; here the average grade of thyroid transplants is approximately 2.6. Transplantations from strain A to strain B and *vice versa* represent therefore typical homoiotransplantations in groups of white rats which do not represent different varieties (as would for instance white, cream and hooded rats). There is no marked difference in this group of experiments between the results obtained with animals of the 37th and 38th generation on the one hand and with animals of the 46th and 47th generation on the other hand, although the average grade in the later generation is slightly lower; but the difference between subseries I and II is so small that it probably falls within the range of probable error.

## Discussion and Conclusions

In reviewing this series of transplantation as a whole, the outstanding result established is the great difference which exists between transplantation in inbred rats and inbred guinea-pigs. In the latter we had found a very marked effect of inbreeding on the individuality differential, while in the case of the inbred rats, this is either absent altogether or is very slight indeed. A comparison between the average grades obtained in non-inbred families and in inbred families of rats is of interest in this connection. However, before comparing these figures, it is well to recall that in grading the intensity of reactions against various tissues and organs there are distinct differences in the absolute intensities of reaction; thus ovarian transplants are on the whole better preserved than, for instance, thyroid transplants and in the former the lymphocytic reaction is rather mild; uterus also is relatively resistant, although the lymphocytic reaction may be intense. Cartilage itself is very resistant, but the surrounding fat and areolar tissue shows distinct

reactions. On the other hand, such tissues as liver and also spleen and bone marrow are very sensitive while thyroid and parathyroid hold a somewhat intermediate position; the latter organs are therefore the best indicators of the intensity of reaction on the part of the host. But while these differences in absolute intensities of reaction between tissues and organs exist, there is an unmistakable correspondence in the relative intensities in reaction found if into the same host different tissues or organs from the same donor are transplanted. Of course, there is in addition a difference in the resistance of the various constituents of the same organ. These observations which one of us has made in previous investigations were again confirmed in these experiments.

If we compare first the grades in ordinary homoiotransplantation with the grades obtained in transplantation from inbred strain A to inbred strain B or *vice versa*, we find in the latter the average grade is 2.57, while in the former the grades are 2.5 in the first and 2.7 in the second series. This indicates practical identity in the breadth of variation in the individuality differential in ordinary homoiotransplantation and in transplantation from strain A to B or *vice versa*. This result might be expected inasmuch as while many generations ago the ancestors of strains A and B belonged to the same litter they have been kept separate through many generations of inbreeding restricted to each strain. If we now compare the average grades of transplants within the inbred strains A and B with those from strain A to strain B or *vice versa* or with the grades of ordinary homoiotransplants, we find that the former are slightly higher, but the differences are relatively so small that they may come within the range of error. Thus the average grade for transplantation within strain B is 2.92, while the average grade for homoiotransplantation in series II is 2.7. This would indicate that through long continued inbreeding it is not possible to produce a decidedly greater similarity between the individuality differentials than is found in the case of ordinary white rats. This conclusion is confirmed in comparing the average grades from brother to brother transplantation in ordinary rats and in inbred rats. In ordinary rats the average grade is 4.66 to 4.75, while in inbred rats (strain A to B combined) it is 4.03. In both cases the average similarity between the individuality differentials of brothers is much greater than that between ordinary rats not belonging to the same litter. Again the averages in the grades

of both inbred and non-inbred rats belong to the same order but this time the average grade of brother to brother transplantation in non-inbred families happens to be slightly higher than in inbred families. We may again, therefore, conclude that the long continued inbreeding through successive brother and sister matings does not result in a greater homogeneity of the individuality differentials of the various members of the same inbred strain.

If we compare the average grades in the 37th to 40th generation with the grades in the 46th and 47th generation we find some interesting differences. Thus in Series A and B the figures for the former are 3.5 and 3.0 and for the latter 1.12 and 2.8 respectively. In brother to brother transplantations within the inbred strains the average grade for the former is 4.78 and for the latter 2.63. This would indicate that instead of decreasing the intensity of the reactions on the part of the host against the transplants, as might have been expected, on the contrary an increase in the intensity of the reaction took place the longer the inbreeding continued. But in appraising these differences we must consider the fact that the number of experiments carried out with the later generations is as yet rather small, and additional experiments are necessary to determine whether this difference is real.

However this latter point may be decided, it is certain that through long continued inbreeding in rats no appreciable approach to homogeneity of the individuality differentials, such as was to a certain extent accomplished in the guinea-pigs, has been reached. This result was not foreseen; as a matter of fact the literature cited on this subject led one to expect the opposite result. As to the causes of differences of behavior of inbred rats and guinea-pigs, one might consider the fact that while in the guinea-pig the mating between brothers and sisters was of a non-selective character, in the case of the rat the strongest individuals were chosen. Thus in the rat a marked deterioration of the stock was prevented, while in the guinea-pig there were definite indications of a deterioration in the characteristics of the inbred animals. Coincidently with deterioration in stock in the case of inbred guinea-pigs the individuality differentials become more and more similar, while with the preservation of the full vigor of the stock in the case of inbred rats the individuality differentials remains dissimilar. We may assume that while the weakest members of the family showed the greatest similarity between the

individuality differentials the strongest individuals were in each case those in which the individuality differentials were most dissimilar. Though this assumption seems plausible, yet in view of the behavior of the most recent inbred generations of rats in which the reactions were especially severe, we must take into consideration the possibility that in addition other, so far unknown, factors may come into play.

## Summary

1. We have analyzed in these investigations in the rat the effect of long continued inbreeding by means of successive brother and sister matings on the constitution of the individuality differentials in inbred strain A and inbred strain B and we compared the results obtained with those obtained previously in various kinds of homoio-, syngenesio- and in autotransplantation in non-inbred rats. In order to be able to compare the results in the various series, we used the same system of grades which we had previously employed in our investigation in non-inbred guinea-pigs and rats and subsequently also in inbred guinea-pigs. The following list of the most important average grades obtained in the various series of experiments give the main results obtained by us.

Non-inbred rats: Autotransplantation, 6. Homoiotransplantation, Series I, 2.7; Series III (transplantation into different varieties), approaching 1.

Inbred rats: Exchange of tissues between animals belonging to different litters. *Strain A*. Subseries I (37th and 38th generations), 3.4. Subseries II (46th and 47th generations), 1.12. Total average for strain A, 3. *Strain B*. Subseries I (37th and 38th generations), 3.0 (3.1). Subseries II (46th and 47th generations), 2.8. Total average for strain B, 2.92. Total average grade of strains A and B slightly below 2.96. Transplantation from strain A to strain B, 2.61. Transplantation from strain B to strain A, 2.53.

Non-inbred rats: Brother to brother transplantation, 4.66 to 4.70.

Inbred rats: Brother to brother transplantation, strain A and B, 40 to 42 generations, 4.78. Brother to brother transplantation, strain A and B, 46th to 47th generations, 2.63. Total average of brother to brother transplantations, 4.03.

2. We may conclude from these results that inbreeding of rats through successive brother and sister matings through a range ex-

tending between 37th and 47th generations does not lead to a distinct diminution in the intensity of the reaction against the transplant if transplantation occurs within the inbred families; at most there may perhaps be a very slight diminution. This holds good also for brother to brother transplantations; while the reactions in these latter experiments are much less marked than in the case of ordinary homoiotransplantations, they are at least as severe in the inbred as in the non-inbred rats.

3. These data indicate that in contrast to the results obtained in inbred guinea-pigs, where inbreeding led to a marked decrease in the intensity of the reaction and where the individuality differentials became very similar (although not yet identical through inbreeding), in the rat successive brother and sister matings, continued through many generations, do not lead to a marked increase in the similarity of the individuality differentials in the inbred animals.

4. This result may be due to selection of the strongest individuals of a litter for mating, the individuality differentials being perhaps most dissimilar in constitution in the strongest individuals. There is, however, the possibility that other not recognized conditions may help to bring about this result. This is at least suggested by the greater severity of the reaction against the transplants in the later as compared with the earlier generations of inbred rats. Further experiments must be made to determine whether this increased intensity in reaction is a constant phenomenon, or is merely accidental.

5. Our previous observations as to the differences in the severity of the reactions against different kinds of tissues and organs were confirmed in this series; we also confirmed our former conclusion that while the absolute severity of the reactions differs according to the relations between the individuality differentials of host and transplant, the relative severity is the same in all the organs tested so far and depends mainly upon the character of the individuality differentials.

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# PYORRHEA ALVEOLARIS: THE RÔLE OF CERTAIN MICROÖRGANISMS FOUND IN THE LESIONS*

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This investigation was undertaken to study the probable significance of *Endamoeba gingivalis*, spirilla, *Bacillus fusiformis* and streptococci found in the lesions of pyorrhea alveolaris. Direct smears were examined in 201 unselected cases of pyorrhea and in seventeen control cases showing normal-appearing gums and teeth, free from pyorrhea. Cultures were made from thirty cases of pyorrhea and also from the seventeen control cases.

## Literature

The literature concerning bacterial factors in pyorrhea is extensive. This report will refer only briefly to some of the more recent investigations.

*Amebas. Endamoeba gingivalis* has been the subject of investigation by Smith and Barrett,[1] Bass and Johns,[2] Sandford and New,[3] Hecker,[4] Keilty,[5] Mitchell, Culpepper and Ayer [6] and others. There is insufficient evidence, it seems from these studies, to establish the pathogenicity of the ameba. Glynn [7] summarizes the evidence against the pathogenicity of this ameba. However, Bass and Johns [2] believed it to be the specific cause of pyorrhea alveolaris. Keilty [5] describes a particular type of gingivitis, apparently of infrequent occurrence, in which, he believes, the *Endamoeba gingivalis* is the exciting cause.

*Spirilla and B. fusiformis.* The term spirilla is used in this investigation to include all types of spirilla and spirochetes observed in the mouth cavity. The present classification of spiral forms is made largely on the basis of morphology and motility. Noguchi [8] has described a mucin-producing spirochete which he isolated from pyorrhea lesions and to which he attributes, in part, the strong fetid odor of pyorrhea. Glynn [7] regards the rôle of spirochetes in pyorrhea lesions as still an open question.

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The fusiform bacillus is a large, pleomorphic, often irregularly staining, gram-negative bacillus with pointed ends. According to Keilty [5] and Glynn [7] it is encountered in practically every mouth. Tunnicliff [9] believes the fusiform bacillus and the associated spirillum to be different stages in the life cycle of the same organism. This view is not in accordance with the observations of Krumwiede [10] and others.

*Streptococci.* Almost all investigators agree that hemolytic streptococci are rarely associated with the lesions of pyorrhea alveolaris. In connection with the study of *Streptococcus viridans* in relation to peridental infections the investigations of Goadby,[11] Gilmer and Moody,[12] Hartzell and Henrici [13] are worthy of careful consideration. Goadby established rather conclusively, it seems, the rôle of dental infections in the etiology of arthritis deformans. Gilmer and Moody found a hemolytic streptococcus in acute alveolar abscesses and *Strep. viridans* in the chronic. Hartzell and Henrici conclude that streptococci belonging to the viridans group are constantly present in peridental infections. In no case did they encounter hemolytic streptococci. They report 29 strains of *Strep. viridans,* 14 of which belong to *Strep. mitis* group, 10 *Strep. salivarius* and 1 *Strep. fecalis* (Andrewes and Horder),[14] the remaining 4 being unclassified at that time. Price [15] reports, in sixty-seven successive cases showing peridental lesions, the finding of many types of streptococci and that *Strep. fecalis* forms 65.5 per cent of these different types. These conclusions differ widely from those of other workers.

## Material and Methods

*Direct Smears.* Material was obtained from 201 unselected cases of clinically positive pyorrhea occurring in patients at the Victoria Hospital and the Ontario Hospital, London, Ontario.

With a wooden applicator, sharpened to a thin chisel-like end and sterilized, material was obtained in each case from the space (gingival) between the gum and the root of the tooth showing the greatest involvement. In most instances this was the labial surface of incisors. The smears were air-dried, gently fixed in the flame and then stained by the method employed by Bass and Johns.[2] While this stain gives rather poor differentiation of the various constituents of the ameba cell, it stains bacteria and pus cells well. Thionin re-

commended by Dupray [16] as a diagnostic stain in pyorrhea proved unsatisfactory as did also Wright's blood stain. The stained smears were carefully examined for amebas, *B. fusiformis*, spirilla and pus cells. The identity of the amebas was further established in a number of cases by warm-stage examination of fresh pyorrheal pus in normal saline. In several instances dark-field illuminations of fresh material were made to study the morphology of the spirilla and their activity. Direct smears from a number of cases were further studied by a special silver nitrate method which Gilbert and Bartels [17] used in the examination of dry smears for *Treponema pallidum*. I have obtained excellent preparations by this method applied to the staining of spirilla in dry smears from pyorrhea lesions.

*Cultures from Pyorrhea Cases*. The determination of streptococci in pyorrhea lesions was made by cultural methods in thirty cases. For this purpose aerobic blood agar plates, made with plain meat infusion agar, adjusted to pH 7.6, plus 5 per cent defibrinated sheep blood, were used. The selected tooth together with its adjacent gum, buccal mucous membrane and immediate surrounding area was thoroughly cleansed with sterile applicator and cotton and 50 per cent alcohol. Wherever possible labial surfaces of lower incisors were used because in this region saliva did not run down and contaminate the field. Under careful precautions material was obtained from a deep portion of the lesion by means of the sterile sharpened applicator, previously mentioned. This material was immediately planted on a blood agar plate by parallel streaks on the surface. From this plate suspected streptococcus colonies were picked to blood agar slants for identification and further study. After twenty-four hours' incubation, the fermentation reactions of the isolated strains were tested on the carbohydrates— lactose, mannite, salicin, saccharose, raffinose and inulin and they were grouped and subgrouped according to Brown.[18] To differentiate between *Strep. viridans* and the pneumococcus, likewise a green-producer, a bile solubility test was done in all cases. Later in the investigation, several cultures were made from pyorrhea lesions on rabbit blood agar for hemolytic influenza bacilli.

*Control Cases*. As controls, direct smears and cultures were made and studied by technic similar to that employed in clinically positive cases, from seventeen young medical students with normal-appearing gums and teeth free from pyorrhea.

## Results and Observations

*The Occurrence of Amebas, Spirilla and Fusiform Bacilli.* In the 201 cases diagnosed as pyorrhea alveolaris, 192 (95.5 per cent) showed the presence of amebas. These are shown in Fig. 1. All positive cases examined showed the presence of *B. fusiformis* and spirilla (Fig. 2) in large numbers and pus cells (Fig. 3) were invariably present, usually in large numbers. The spirilla are of more than one type. They vary greatly in number, width and depth of their curves and in length. This variation is well shown in Fig. 4.

In the series of seventeen clinically normal gums, amebas were absent in all cases. *B. fusiformis* and spirilla, while in much smaller numbers than in the pyorrhea cases, were present in all of the seventeen normal gums.

*Cultures from Pyorrhea Cases.* The accompanying table shows the results of the cultures for streptococci in pyorrhea cases. In these thirty cases it will be seen that in every case green-producing streptococci (*Streptococcus viridans* [Schottmüller]) were present. *Strep. viridans* was present in large numbers and in all cases it formed decidedly the majority of organisms present on the plates. In several instances the cultures on blood agar plates, obtained as above described, showed a pure growth of *Strep. viridans*. From the fermentation reactions these belong to two groups namely *Strep. mitis* and *Strep. salivarius* (Andrewes and Horder), nineteen of which are *Strep. mitis* and eleven *Strep. salivarius*. Examination of the accompanying table will readily show the subgroups (according to Brown) to which these belong and the number in each subgroup. In no instance were hemolytic streptococci present.

In no case was *Strep. fecalis* found while Price [15] reports that 65.5 per cent of the different types of streptococci, found by him in peridental lesions, belong to the *Strep. fecalis* group. He also reports the finding of *Strep. hemolyticus* as well as *Strep. viridans*. In these two important respects the results of this study differ from those of Price.

All the cultures made on rabbit blood agar for the hemolytic influenza bacillus failed to show the presence of this organism.

*Cultures from Normal Controls.* In the seventeen cultures obtained from the gums of the normal controls *Strep. viridans* was present in only six instances. Of the remaining eleven cultures, ten

showed no growth on the blood agar plates after seventy-two hours incubation while the eleventh showed only two colonies both of which were organisms other than *Strep. viridans*. All of the six cultures, showing *Strep. viridans*, presented a striking diminution in the number of streptococci as compared with those cultures from pyorrhea cases while one of these six showed one lone streptococcus colony. Of the six strains of *Strep. viridans* isolated in this normal

TABLE SHOWING THE RESULTS OF DEEP CULTURES FROM GINGIVAL SPACE FOR STREPTOCOCCI. THIRTY CASES OF PYORRHEA ALVEOLARIS AND SEVENTEEN NORMAL CONTROL CASES

| | Groups of *Strep. viridans* | Subgroups (according to Brown [18]) | | | | |
|---|---|---|---|---|---|---|
| | | .1 | .2 | .3 | .4 | Total |
| Positive pyorrhea | *Strep. mitis* | 8 | 5 | 5 | 1 | 19 |
| | *Strep. salivarius* | 5 | 5 | 0 | 1 | 11 |
| *Normal gums | *Strep. mitis* | | | | | 0 |
| | *Strep. salivarius* | 2 | 4 | 0 | 0 | 6 |

* 11 of the 17 cultures from normal gums showed no streptococci.

series all belonged to the group *Strep. salivarius*. (For subgroups see table.) In no case of this series was *Strep. hemolyticus* encountered.

*Sections of Teeth and Gums from Pyorrhea Cases.* Specimens of teeth, gums, alveolar process and adjacent jaw showing well marked pyorrhea lesions, in a number of cases, were removed at necropsy and decalcified. From these, sections were prepared and stained by hematoxylin and eosin and by MacCallum's technic [19] for bacteria in tissues. From careful examination of these sections it is seen that the ameba does not invade the tissues of the gum. Throughout the stroma of the gum adjacent to the tooth involved, there is a well marked chronic inflammatory reaction with a preponderance of plasma cells (Fig. 5). In the sections stained with MacCallum's stain there are large numbers of spirilla, fusiform bacilli and gram-positive cocci situated rather superficially in the necrotic material and debris in the gingival space. However, situated deeper, in the peridental membrane beyond the necrotic margin, are a few gram-

positive cocci arranged in pairs and short chains. These have the morphology of the streptococcus.

## Discussion

*Significance of Amebas, Spirilla and Fusiform Bacilli.* It is probable that the ameba should be regarded as nonpathogenic and harmless. In so far as has been observed it does not invade living peridental tissues. When present in pyorrhea pus it occurs in relatively very small numbers as compared with the pus cells and bacteria present. It seems to be an expression of an unclean mouth, the necrotic material and debris being the habitat of the ameba, because in this series they do not occur in clean normal mouths. In my experience the more unclean the mouth the greater is the probability of finding the ameba. All animal experiments carried out by other investigators with this ameba have failed to produce pathologic lesions.

From a study of the literature it appears that spirilla and fusiform bacilli have received little recognition as important etiologic factors in pyorrhea alveolaris. Kline [20] has reported seven cases of spirochetal pulmonary gangrene associated with severe dental caries as a primary focus and referred to seventeen other cases reported in the literature. As previously stated, spirilla and fusiform bacilli occurred in all normal cases examined as well as in pyorrhea cases, they do not invade living peridental tissues and they occur in large numbers in smears obtained superficially. It may, therefore, be concluded that they are probably of little pathologic significance in pyorrhea.

*Rôle of Streptococcus viridans in Pyorrhea Lesions.* The pathogenicity of the *Strep. viridans* group is well known and has been established by workers mentioned in this paper and by others. In this study *Strep. viridans* was constantly present in pyorrhea lesions and absent in eleven of seventeen normal gums. It may be that some of the supposedly normal cases showing *Strep. viridans* are in reality early incipient cases of pyorrhea which, at this stage, are not recognizable clinically. This organism is a constant inhabitant of the normal buccal cavity. Zinsser,[21] in discussing the bacteria in the normal mouth and pharynx makes the following statement: "Of the streptococci the Viridans is almost always present. The isolation of a 'viridans' from inflammatory processes of the mouth

and throat, therefore, has very little true significance unless it is isolated from a closed process, such as a tooth abscess, or unless other strong corroborative evidence can be adduced." However, fully recognizing the significance of this statement, by the technic employed in this investigation I believe that I have excluded contamination from the surrounding surfaces. The fact that ten of the seventeen cultures from normal controls showed no growth on aerobic blood agar plates seems to indicate the relative efficiency of the technic. With the thorough preliminary cleansing and by the method previously described, material obtained under these circumstances from a deep portion of the pyorrhea lesion would be almost equivalent to that obtained from a closed process. It is important in cultures obtained in this manner to take into consideration relative numbers, if different types are present. In pathologic lesions it is usually logical to attach importance to the predominating type. It is interesting to note that 78 per cent of the streptococci present in normal saliva are of the *Strep. salivarius* type (Glynn [7]) while 63 per cent of the *Strep. viridans* strains in my series isolated from pyorrhea cases belong to the *Strep. mitis* group.

The constant association of *Strep. viridans* in large and predominating numbers with pyorrhea, as shown by cultural methods, its absence in the majority of normal gums cultured and the occurrence, in sections of pyorrheal tissue, of a gram-positive coccus morphologically a streptococcus in the deeper peridental tissues, may be taken as evidence in favor of this organism being of much importance in pyorrhea.

That the production of pyorrhea alveolaris should be due to a single factor seems highly improbable. It may be that a combination of factors, such as nutritional disturbances, traumatic influences (mechanical and chemical) and increasing age, initiates the condition and a bacterial infection follows. Since pyorrhea alveolaris is a suppurative lesion, *Strep. viridans*, essentially a nonpyogenic coccus, is not likely the exciting cause of this pathologic condition. However the constant presence, in large numbers, of *Strep. viridans* in deep portions of the lesion, its known pathogenicity, its invariably constituting the majority of organisms in cultures on blood agar plates and its invasion of living peridental tissues, present evidence that it is a grave menace in a primary focus for the production of certain systemic diseases.

In the greater number of cases more than one type of *Strep. viridans* may be present in individual cultures from pyorrhea lesions, although I have found that this is not always the case. If two or more types be present, usually one type is decidedly predominating. The results here reported were determined by fishing from each culture a representative of the predominating type of *Strep. viridans* colony.

The pathogenicity of different strains of *Strep. viridans* isolated from pyorrhea lesions was tested upon rabbits with varying results. Some strains were apparently nonpathogenic. Others killed the animals within twenty-four hours. One strain of *Strep. mitis* produced a fatal vegetative endocarditis, strikingly similar to that found in human cases dying of *Strep. viridans* endocarditis.

In a number of cases I have found that the blood serum of patients showing well marked pyorrhea lesions contains agglutinins for the predominating strain of *Strep. viridans* found in their lesions.

Further observations are now in progress upon variations in the types of *Strep. viridans* encountered in individual cases, pathogenicity, toxicity and agglutination reactions. These will be reported later if results seem to justify publication.

## Summary and Conclusions

1. *Endamoeba gingivalis* was present in direct smears in 95.5 per cent of 201 pyorrhea cases examined. Evidence is here submitted in support of the belief that this ameba is a harmless parasite. It was not found in the clean, normal gums examined.

2. *B. fusiformis* and spirilla were found in the smears from all of the cases of pyorrhea alveolaris studied and were also present in each of the seventeen normal gums. For reasons here given they are probably of little significance in the production of the pyorrhea lesion.

3. In making cultures a special technic was employed in an attempt to exclude contamination from saliva, buccal mucosa, adjacent teeth, gums and other sources.

4. *Strep. viridans* was present in each of thirty cases of pyorrhea examined culturally. In all instances it was decidedly the predominating organism present on the blood agar plates. The isolated strains belong to two groups, *Strep. mitis* and *Strep. salivarius* respectively, and seven subgroups (table). In seventeen normal gums

studied *Strep. viridans* was absent in eleven. Strains of this organism in the remaining six belong to one group, namely *Strep. salivarius* of which there are two subgroups (table). For reasons stated, *Strep. viridans* is probably of much importance in pyorrhea.

5. Hemolytic streptococci were not present in any of the cases examined culturally nor were hemolytic influenza bacilli.

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## DESCRIPTION OF PLATE

### Plate 56

Fig. 1. Three amebas (*Endamoeba gingivalis*, Gros) stained with carbol fuchsin and methylene blue. The dark inclusion-bodies and vacuoles are clearly shown. The nucleus is poorly defined with this strain. × 1000.

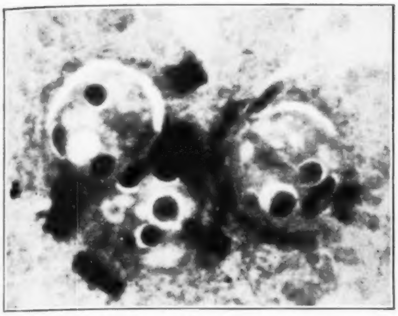
Fig. 2. Spirilla and fusiform bacilli stained with carbol fuchsin and methylene blue. Note the bands of irregular staining in the fusiform bacilli. × 1000.

Fig. 3. Polymorphonuclear leucocytes. This shows the acute suppurative nature of the pyorrhea lesion and gives an idea of the large numbers of pus cells encountered. × 1000.

Fig. 4. Spirilla stained by Fontana's method. Different types of spirilla demonstrating the variation in morphology are here shown. × 1200.

Fig. 5. Hematoxylin-eosin section of decalcified tooth and gum showing well marked pyorrhea alveolaris. This section shows a pronounced chronic inflammatory reaction with plasma cells predominating and occasional polymorphonuclear leucocytes. The dentine appears at the extreme left of the figure. The clear area is the gingival space. × 650.

Note: — All figures are photomicrographs of material obtained from pyorrhea cases. Figs. 1 to 4 inclusive, Zeiss apochromatic 2 mm. objective, compensating × 10 ocular. Fig. 5, 4 mm. objective, × 10 ocular. Wratten M plates were used for all figures.



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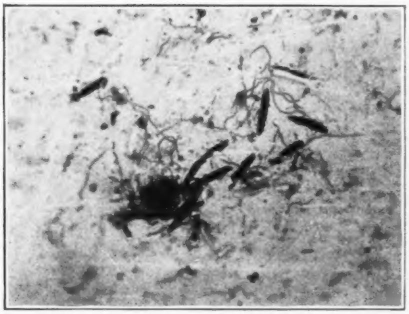

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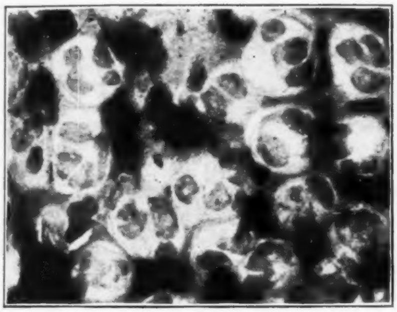

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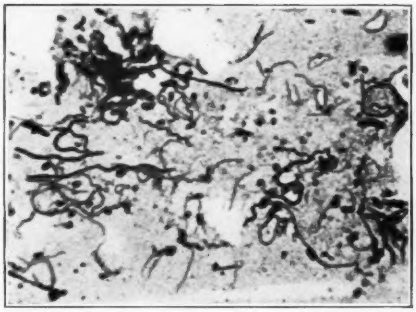

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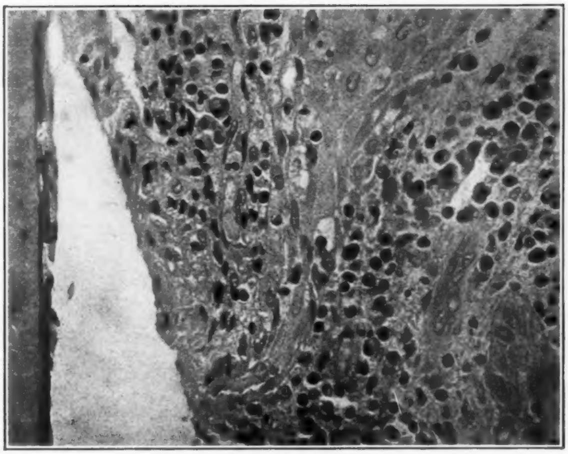

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